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(54) Title: A NEW PHARMACEUTICAL USE OF FUSIDIC ACID AND DERIVATIVES THEREOF

(57) Abstract

The use of fusidic acid or a derivative thereof for the manufacture of a composition for interfering with cytokines in a biological system, in particular for interfering with a cytokine such as a lymphokine, interleukine, monokine, interferon or colony-stimulating factor. Fusidic acid and derivatives thereof are useful for preventing effects of cytokines known to be pathogenetically involved in pathological conditions. Said conditions may e.g. be: insulin-dependent diabetes mellitus (Type 1), hyper- or hypofunctioning of the thyroid gland (e.g. thyroiditis), Addison's disease, endogenous uveitis, eye surgery such as cornea transplantation, cataract operation and laser surgery, transplant rejection, graft-versus-host disease, Crohn's disease or ulcerative colitis, pernicious anemia, celiac disease, contact dermatitis, allergic/atopic dermatitis, pemphigus vulgaris or pemphigoid, multiple sclerosis, systemic lupus erythematosus, polymyalgia rheumatica, conditions related to vasculitis phenomena, neoplastic diseases such as multiple myeloma, septic shock caused by gram-negative bacteria, DIC, arteriosclerosis, periodontal diseases, arthritis, osteoarthrosis or arthritis urica.

A NEW PHARMACEUTICAL USE OF FUSIDIC ACID AND DERIVATIVES THEREOF

The present invention relates to a new pharmaceutical use of fusidic acid and derivatives thereof, in particular the use for prevention and/or treatment of diseases, the pathogenesis of which is related to the production and/or function of certain immunoinflammatory mediators, especially cytokines. In particular, the invention relates to the use of fusidic acid and derivatives thereof and to the prevention and/or treatment of diabetes mellitus (type 1) (insulin-dependent diabetes mellitus (IDDM)), thyroid diseases, rheumatoid diseases, inflammatory diseases of the gut, skin diseases, conditions related to transplant rejection, endogenous uveitis and conditions related to opthalmological interventions.

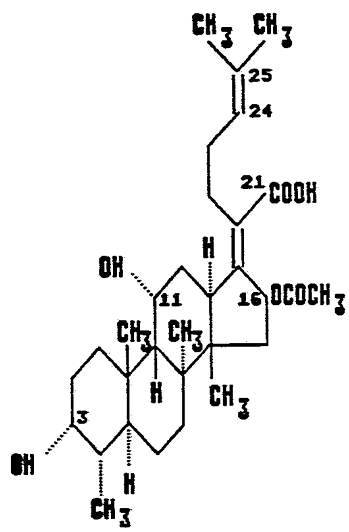
BACKGROUND OF THE INVENTION

There is increasing clinical and experimental evidence that many autoimmune diseases develop as as result of abnormalities in the immune system, especially in T lymphocyte-mediated immunity. Many signs and symptoms of infectious, inflammatory and neoplastic diseases evolve as a result of stimulation of cellular immunity. In addition, although immunocompetent cells may not necessarily be involved at the initial stage, abnormal regulation of otherwise appropriate cellular immune reactions may lead to acute and chronic diseases. These diseases are generally of unknown etiology and include systemic rheumatic diseases (e.g. rheumatoid arthritis (RA)), organ-specific endocrine diseases (e.g. insulin-dependent diabetes mellitus (IDDM)) and inflammatory diseases of the gut (e.g. Crohn's disease) and skin (e.g. contact dermatitis). Disorders of cell-mediated immunity, however, contribute to many other immunoinflammatory and proliferative diseases as will be described in further detail in the following.

The treatments available in relation to said diseases are usually symptomatic or palliative treatments, i.e. most of the drugs prescribed in connection with said diseases are directed at the allaying of symptoms and usually have no curative effect. Other treatments are so-called substitution therapies which involve life-long supplying to

the patient of substances, e.g. hormones, needed due to a reduced/insufficient internal production of said substance. Said treatments are often unsatisfactory and imply unwanted and often serious side-effects. Thus, improved methods of treatments and improved pharmaceutical compositions are needed.

Fusidic acid and certain derivatives thereof can be isolated from the fermentation broth of certain strains of Fusidium coccineum and have for a number of years been known as efficient and pharmaceutically acceptable antibiotics. Fusidic acid, 16-(acetyloxy)-3,11-dihydroxy-29-dammara-17(20),24-dien-21-oic acid; (3α,11α,16β-trihydroxy-29-nor-8α,9β,13α,14β-dammara-17(20),24-dien-21-oic acid 16-acetatate), is a tetracyclic triterpenoic acid with a steroid-like primary structure of formula I:



I

It is well-known that fusidic acid inhibits bacterial protein synthesis and it may be bacteriostatic and/or bactericidal. It is especially active against Staphylococcus aureus, including strains that are resistant to the penicillins or to other antibiotics. The drug is used clinically both in its acid form, and as the sodium and diethanolamine salts. The sodium salt of fusidic acid has the actions and uses of fusidic acid. It is more soluble in water, and is readily absorbed from the gastro-intestinal tract. It is also used in topical preparations. For severe staphylococcal infections the antibiotic may be given intravenously as e.g. diethanolamine fusidate. A description of chemical and microbiological modifications of fusidic acid is given in Structure-Activity Relationship in Fusidic Acid-Type Antibiotics (45), and fusidic acid and derivatives thereof are described

in Pharmacopoieas and in Martindale, The Extra Pharmacopoeia, 28th edition (1982).

Fusidic acid and derivatives thereof are described in PCT application No. WO 86/03966, published examined Danish application No. 148390 (US 4,119,717), DK 96349 (GB 930786), DK 99802 (AT 252446), DK 101687 (US 3,334,014), DK 104399 (US 3230240), DK 105981 (US 3,230,240), DK 107349 (GB 999794), DK 116940 (US 3499012), DK 131348 (US 3,867,413), DK 142852 (US 4100276, US 4162259 and US 4315004), U.S. Patent Nos. 3,376,324, 3,629,300, 3,920,817, 4,004,004, 4,025,620, 4,060,606, 4,315,004 and U.K. Patent Nos. 2,093,348 and 987,042. However, none of these prior art documents are related to the use of fusidic acid or functional derivatives thereof for interfering with specific immunological response mechanisms or for interfering with cytokines (lymphokines, interferons, interleukins, colony-stimulating factors and/or monokines), nor related to methods for treating conditions related to disturbances in the cytokine system. Occasional mentioning in the medical literature of specific patients, e.g. diabetics, receiving a short transitory fusidic acid medication due to a bacterial infection is obviously irrelevant to the teaching and embodiments of the present invention.

SUMMARY OF THE INVENTION

According to the present invention it has been found that fusidic acid and derivatives thereof can be used for the prevention and/or treatment of certain forms of inflammatory diseases or processes, epecially forms related to the immune and/or hormone system. It is contemplated (as described in detail in the following description of immunological mechanisms) that the action mechanism is via interference with the action of mediators of the immune system, in particular cytokines such as monokines and lymphokines, i.e. that fusidic acid interferes with/suppresses the action of certain cytokines and thus inhibits pathological processes leading to tissue damage.

In its broadest aspect the present invention relates to the use of fusidic acid or a derivative thereof for substantially inhibiting a biological effect related to a cytokine such as a lymphokine, inter-

leukin, monokine, interferon or colony-stimulating factor for the prophylaxis or treatment of a condition related to a disturbance of a cytokine system such as the lymphokine, interleukin, monokine, interferon or colony-stimulating factor system. The broadest aspect of the invention also relates to a method of treating in a human a condition related to a disturbance in a cytokine system which method comprises administering to the subject an effective amount of fusidic acid or a functional derivative thereof. Thus, the use according to the present invention is *not* based on the use of fusidic acid or functional derivatives thereof as antibiotics.

DETAILED DESCRIPTION OF THE INVENTION

General information on cytokines and immunoinflammatory diseases

Many signs and symptoms of infectious, inflammatory and neoplastic diseases evolve as a result of stimulation of cellular immunity. In addition, although immunocompetent cells may not necessarily be involved at the initial stage, abnormal regulation of otherwise appropriate cellular immune reactions may lead to acute and chronic diseases. These diseases are generally of unknown etiology and include systemic rheumatic diseases (e.g. rheumatoid arthritis (RA)), organ- specific endocrine diseases (e.g. insulin-dependent diabetes mellitus (IDDM)), and inflammatory diseases of the gut (e.g. Crohn's disease) and skin (e.g. contact dermatitis). Disorders of cellmediated immunity, however, contribute to many other inflammatory and proliferative diseases (see Table 1).

TABLE 1

Some diseases where macrophages/T-lymphocyte-mediated immune reactions are considered pathogenetically important.

Atopic dermatitis and contact dermatitis
Psoriasis and psoriatic arthritis
Mycosis fungoides, Sézary syndrome and other T lymphocyte proliferative disorders
Pemphigus vulgaris and pemphigoid
Erythema nodosum
Scleroderma
Uveitis
Bechet's disease
Sarcoidosis Boeck
Sjögren's syndrome
Rheumatoid arthritis
Juvenile arthritis
Reiters syndrome
Gout
Osteoarthrosis
Systemic lupus erythematosis
Polymyositis and myocarditis
Primary biliary cirrhosis
Crohn's disease
Ulcerative colitis
Multiple sclerosis
Aplastic anemia
Idiopathic thrombocytopenic purpura
Multiple myeloma and some B cell lymphomas
Multiple sclerosis and other demyelinating diseases
Simmonds' panhypopituitarism
Graves' disease and Graves' opthalmopathy
Subacute thyreoditis and Hashimoto's disease
Addison's disease
Insulin-dependent diabetes mellitus (type 1)

Miscellaneous
Various clinical syndromes with vasculitis (e.g. polyarteritis nodosa, Wegener's granulomatosis, giant-cell arteritis, fever, malaise, anorexia (e.g. in acute and chronic inflammatory and infectious diseases)
Disseminated intravascular coagulation (DIC)
Arteriosclerosis
Shock (e.g. in gram negative sepsis)
Cachexia (e.g. in cancer, chronic infectious and chronic inflammatory diseases)
Transplant rejection and graft-verus-host disease

T lymphocytes govern the induction and regulation of cell-mediated immune reactions, and proteins and glycoproteins produced by T lymphocytes (lymphokines) initiate and control the immune response (1,2). However, antigen activation of T cells requires physical interaction with antigen-presenting cells such as macrophages (MØ), and these cells also produce mediator molecules crucial for T cell activation and the subsequent triggering of B lymphocytes to become antibody-producing plasma cells. These mediators are also responsible for the recruitment and activation of many other cell types which build up inflammatory infiltrates in the diseased tissues (1-4).

The lymphokines and the lymphocyte-activating mediators produced by antigen-presenting cells are collectively termed cytokines. Hence, cytokines are essential transmitters of cell-to-cell communication in both physiological and pathophysiological infectious, immunoinflammatory, and growth processes. In many cases they also function as hormones providing information between the immune system and other tissues and organs. Furthermore, many cytokines are produced by cells outside the immune system (e.g. in the skin (5) and in blood vessels), and cytokines may therefore act as autocrine or paracrine hormones without necessarily involving immunocompetent cells.

Cytokines are active at extremely low concentrations (10^{-10} to 10^{-15} M) via binding to specific receptors on a large number of target cells. Most cytokines probably act in the vicinity of the

production site but, as mentioned above, some of the mediators modulate functions of cells at distant sites via blood and lymph circulation. Fig. 1 shows the dynamics of production and function of cytokines during an immune reaction in a vascularized tissue.

Macrophages

Macrophages (MØ) play a central role in the defence against microbial and neoplastic diseases, and they are important in a wide variety of tissue repair processes (2).

Some MØ functions are performed continuously, such as removal of aged erythrocytes. Other functions are performed periodically, and these functions need prior activation of the MØ. The cells may be activated directly, for instance by contact with microorganisms or their products, such as endotoxins (lipopolysaccharides), muramyl peptides, and other cell wall components. This direct means of MØ activation is in higher animals improved considerably by a more indirect form of activation via lymphokines (1-3). Thus, interferon α and -γ (IFNα and IFNγ) are important activators of MØ, but other MØ activating lymphokines exist (1).

Phagocytosis itself and, perhaps more importantly, immunecomplex- or lymphokine-mediated activation trigger the MØ to synthesize and release a number of biologically active molecules, including prostaglandins, proteolytic enzymes, complement components, etc. Four of these factors, interleukin 1α (IL-1α), interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumour necrosis factor-α (TNFα), have far-reaching biological and pathophysiological significance (1-8).

Macrophage-derived polypeptide mediators

The molecular biology of the best characterized human MØ hormones has recently been extensively reviewed (2,4,6-8). They are all members of a growing family of cytokines which includes the interferons and some hemopoietic growth factors. The MØ cytokines are also produced during conditions of "stress", and they are important mediators of fever, the acute-phase reaction, and the generation of cachexia as a result

of chronic infectious, inflammatory, autoimmune, and neoplastic diseases.

Production of IL-1α/β, IL-6 and TNFα

Although IL-1α/β, IL-6 and TNFα are produced primarily by activated MØ, the capacity to elaborate these molecules, particularly IL-1α/β and IL-6, is not confined to MØ (2). Natural killer cells (NK cells) and B lymphocytes are already known to produce IL-1α/β, and fibroblasts were the cells originally shown to produce IL-6 (= IFNβ2). In fact, it is likely that all nucleated cells may produce these peptides, provided they are triggered by an appropriate stimulus.

The mature mediator molecules are single-chain polypeptides consisting of 169 (IL-1α), 153 (IL-1β), 184 (IL-6), and 157 (TNFα) amino acids. Native human IL-6 is glycosylated, and the resulting molecular weight of this cytokine species range between 17 and 28 kD.

The genes for TNFα and TNFβ are both located in the class III region of the major histocompatibility complex, i.e. the LA region in man. Recent studies in our laboratories show that blood mononuclear cells obtained from HLA-DR2 positive individuals exhibit a significantly diminished TNFα response *in vitro* (9). This is important, because the HLA-DR2 type is positively associated with diseases such as multiple sclerosis and narcolepsy and negatively associated with rheumatoid arthritis and IDDM.

Immunological functions of IL-1α/β and IL-6 - clinical relevance

Receptors for IL-1α/β, IL-6 and TNFα have been found on most of the cell types known to respond to the cytokines. IL-1α and IL-1β are thought to bind to the same receptor on T and B lymphocytes, and both peptides are capable of down-regulating the expression of the CD4 structure on human T cells (10). The CD4 structure is directly engaged in antigen presentation, possibly through interaction with MHC class II molecules on antigen-presenting cells (Fig. 1). It is there-

fore possible that the T lymphocyte IL-1 receptor binds to the major membrane-associated IL-1 species (IL-1α) on the antigen-presenting cell, and that this receptor is physically or functionally related to the CD4 molecule (Fig. 1 and ref. 2).

IL-6 has been shown to be identical with B cell-stimulating factor 2 and a factor necessary for the growth of plasmacytomas and B cell hybridomas (2, 7). IL-6 is also a cofactor in T cell activation, probably because it induces IL-2 receptors and functions as a second signal for IL-2 production (2).

Modulation of immune reactions and tolerance induction

It may prove clinically important that interference with the IL-1-mediated "second signal" for T cell activation may lead to antigen-specific tolerance. Thus, triggering the T cell receptor by a properly processed antigen, while at the same time preventing the ensuing IL-1-induced activation of these specifically reactive cells, prevents the selected clone(s) from responding to the same antigen at a later time, both *in vitro* (11) and *in vivo* (12).

Because of the central importance of IL-1α/β and IL-6 in T and B lymphocyte activation, any treatment that blocks the production or the immunological functions of these mediators would be expected to suppress the entire cellular immune system. This may be especially beneficial in the management of patients receiving transplants.

Other functions of IL-1, IL-6 and TNFα - clinical relevance

IL-1α/β, IL-6 and TNFα are pleiotropic in that they exert multiple functions on many different cell types (2). It has become clear that IL-1α/β, TNFα, IL-6 and lymphotoxin (=TNFβ) are identical with the factors originally described as endogenous or leukocytic pyrogens; i.e., hormones of leukocytic origin which produced fever (3). It is not entirely clear, however, whether all these cytokines act in a similar fashion in the brain during fever induction.

Acute-phase reaction

Fever is often part of the acute-phase reaction usually seen in conjunction with acute and chronic infectious and immunoinflammatory diseases, and in cancer (2-4, 8). IL-1α/β and IL6, and to a lesser extent TNFα, induce hepatocytes to synthesize acute-phase proteins, including serum amyloid A, C-reactive protein, fibrinogen, haptoglobin, complement components and clotting factors. The elevated level of fibrinogen, especially if accompanied by anemia, causes an increased erythrocyte sedimentation rate, a commonly used clinical parameter of "inflammation" Since IL-1α/β and TNFα are potent inducers of IL-6 in many cell types, including hepatocytes, it is possible that IL-6 is a second mediator of the acute-phase response elicited by IL-1α/β and TNFα.

Cachexia, disseminated intravascular coagulation (DIC) and shock

The above mentioned clinical picture is often associated with disturbances in carbohydrate-, lipid- and protein metabolism eventually resulting in wasting (cachexia). In rare situations, clotting abnormalities and shock may occur (6).

It was previously thought that microbial products, especially endotoxin from the cell wall of gram-negative bacteria (lipopolysaccharide (LPS)) were directly responsible for these often life-threatening symptoms, if triggered by bacterial infection. This is now known to be incorrect, because LPS is a potent inducer of MØ IL-1α/β, IL-6 and TNFα, and all pathophysiological processes associated with LPS-induced shock can be reproduced by injection of TNFα and to a lesser extent by IL-1α/β (2,6).

TNFα is probably also responsible for other phenomena associated with LPS-induced disease, such as metabolic acidosis, DIC and cachexia (2,6). It is likely that some of these phenomena are mediated through TNFα-induced production of IL-1α/β or IL-6, or one or several other (unknown) hormones. For example, TNFα is a potent inducer of IL-1α/β in endothelial cells, and all three cytokines trigger the release of procoagulant factor(s) from these cells. It is therefore

likely that both mediators may be involved in the pathogenesis of some vascular diseases with or without accompanying clotting abnormalities.

Neoplastic diseases

Unregulated production of IL-6 may be pathogenetically involved in the manifestation of several human diseases, including cardiac myxoma and various lymphoid malignancies such as multiple myelomas and various T and B cell lymphomas (6). It is interesting, that myeloma cells constitutively produce IL-6 and express IL-6 receptors, and that the proliferation of myeloma cells in vitro is inhibited by specific antibodies to human IL-6 (13).

Other proliferative diseases - psoriasis

IL-6 and, possibly, TNFα may also be involved in other proliferative disorders. Using specific antibodies to human rTNFα and human rIL-6, we have recently demonstrated TNFα and IL-6 in skin biopsies (5, 5A). The expression of both mediators was considerably augmented by UV-irradiation, and relatively large amounts of IL-6 and TNFα were found in psoriatic lesions. Interestingly, local treatment of the psoriatic lesions with a vitamin D_3 analogue resulted in clinical improvement, and biopsies performed after treatment showed unaltered expression of TNFα, but an almost complete elimination of the expression of IL-6 in the upper epidermal layers. It is therefore possible that IL-6 may be involved in the uncontrolled proliferation of keratinocytes seen in psoriasis.

Rheumatic diseases

IL-1α/β, IL-6 and TNFα have many biological effects which qualify them as important in the pathology leading to rheumatic diseases (2,8). The most relevant actions of IL-1α and -β are most likely their stimulatory effects on T and B cells (nonspecific production of inflammatory lymphokines and immunoglobulins), MØ (e.g. MHC class II antigen expression and production of eicosanoids), chondrocytes (production of collagen type II), fibroblasts (production of collagen

types I and II and eicosanoids), and osteoclasts (bone resorption). TNFα and IL-6 share many of these effects with IL-1, although IL-6 does not cause bone resorption (Pedersen JG & Bendtzen K, unpublished).

Several cellular sources of the cytokines are present in the arthritic joint, including MØ, B lymphocytes, fibroblasts and vascular endothelial cells (2,8). The demonstration of IL-1α/β in synovial fluids from rheumatoid and osteoarthritic joints further suggests that IL-1 in particular may play an important role in the pathogenesis of arthritis and, possibly, in osteoarthrosis (2, 14). An unbalanced reaction between immunocompetent cells and accessory cells such as MØ, NK cells and polymorphonuclear leukocytes might continually induce IL-1α/β, IL-6 and other inflammatory molecules, for example prostaglandins and leukotrienes. This might contribute to the chronic nature of diseases such as RA.

The arthritogenic activity of IL-1β is most clearly seen from *in vivo* experiments using a rabbit arthritis model (15). Human rIL-1β induces MØ and neutrophil accumulation 24 h after injection in the knee, and this is associated with depletion of proteoglycan from the articular cartilage and an increase in the glycosaminoglycan content of the joint fluid. The same signs of cartilage damage by IL-1β are seen in joints of rabbits previously depleted of polymorphonuclear leukocytes and MØ by systemic administration of nitrogen mustard. This suggests that IL-1β itself is capable of stimulating resident cells of the joint, such as the chondrocytes, to cause proteoglycan depletion. Again, it is possible that some of the effects ascribed to IL-1β may be mediated via local, IL-1β-induced production of other cytokines, particularly IL-6 and IL-1α.

It is noteworthy that urate crystals are potent activators of MØ IL-1 production (16). Thus, IL-1 may contribute to the manifestations of gout.

Insulin-dependent diabetes mellitus (IDDM) and thyroiditis

Recently, the predominant species of human IL-1, IL-1β, was shown to be a potent suppressor of insulin production *in vitro*, possibly as a result of a direct and selective cytotoxic effect on pancreatic islet β-cells (17). IL-1α also reduces insulin production, but it is 10 times less potent than IL-1β. The effect of IL-1β is biphasic because increased insulin production is consistently observed at low concentrations of rIL-1β (10 - 200 pg/ml = $5*10^{-13}$ - 10^{-11} M), whereas higher concentrations reduce both the extracellular and intracellular contents of insulin, probably by a direct and selective damaging effect on the β-cells.

Although TNFα itself fails to affect β-cell function, the suppressive effect of IL-1β is augmented significantly by TNFα (3,8). Other cytokines, by themselves or in conjunction with IL1β, fail to affect β-cell function.

It is interesting that IL-6 augments the release of insulin from normal rat islets and that IL-1α and IL-1β induce IL-6 in the islets themselves (17A). Furthermore, even though IL-6 increases insulin production, this cytokine causes structural changes in β-cells similar to those caused by IL-1 (17A). Thus, local IL-1-induced production of IL-6 may underly the biphasic insulin response observed with various concentrations of IL-1 (17). Furthermore, IL-6, in conjunction with IL-1, may contribute to the immunoinflammatory processes eventually leading to IDDM.

Similar effects as those described above have been obtained when testing IL-1β on human thyroid cells removed from normal thyroid tissue during surgery of paraadenomatous glands (17A, 18). The secretion of thyroglobulin and cAMP is markedly suppressed even by low concentrations of rIL-1β (15 pg/ml = 10^{-12} M). The effect is augmented by TNFα and, in addition, by IFNγ. The thyrocytes are not killed by IL-1β, but their ability to form follicles and accumulate glycogen in response to thyroid stimulating hormone is suppressed (18). Again, a stimulatory effect on thyroglobulin production is consistently observed at very low concentrations of IL-1β (1.5 - 150 fg/ml = 10^{-16} - 10^{-14} M) (18), and IL-1β is a very potent inducer of IL-6 in thyrocytes (17A).

The above findings and considerations indicate a central role of MØ and perhaps NK cells, and their products IL-1α/β, TNFα and IL-6 in a number of physiological and pathophysiological conditions (3,8). Prolonged exposure of cells to increased levels of these cytokines might lead to structural or functional damage to the cells (e.g., in IDDM), for instance if the target cells for unknown reasons are surrounded by cytokine-producing MØ, NK cells and T and B lymphocytes, as is the case in the early stage of IDDM development. Endothelial cells, fibroblasts and other cells in the connective tissue may also contribute in these situations through an inappropriate production of cytokines such as IL-1α/β and IL-6. Moreover, low concentrations of IL-1α/β and IL-6 may accumulate in target tissues by diffusion from the blood during conditions of stress, and the cytokines may therefore have important physiological functions by potentiating the secretion of insulin, thyroid- and, possibly, pituitary- and adrenocortical hormones under these circumstances (Fig. 1).

As described above, the negative association between HLA-DR2 and the production of TNFα may be implicated in some HLA-associated diseases such as IDDM and RA (3,8). In the latter two, HLA-DR2 is associated with resistance to disease, and this might be explained if TNFα is involved, directly or indirectly, in the destruction of islet β-cells in IDDM and of cartilage and bone in RA and osteoarthrosis.

Therapeutic considerations

Considering the many putative pathophysiological functions of IL-1α/β, IL-6 and TNFα, intervention with the production or action of these hormones might be of great benefit in a number of infectious and immunoinflammatory diseases and, possibly, in certain neoplastic diseases. The production of the mediators is prevented by high doses of glucocorticoids, which directly suppress gene transcription and, if the genes have been transcribed, the mobilization of mRNA, at least in the case of IL-1α/β and TNFα (6,8).

The effect of IL-1α/β on T cells *in vitro* is prevented by cyclosporin (=Cyclosporin A) (19), by vitamin D_3 (1,25 $(OH)_2$ D_3) and a synthetic

vitamin D_3 analogue (20). Whether cyclosporin and vitamin D_3 also prevent some of the other activities of IL-1α/β is not completely clear, although their metabolic effect on pancreatic β-cell function is slightly inhibited by both cyclosporin (21) and vitamin D_3 (Buschard K & Bendtzen K, unpublished).

Since cyclosporin has many serious side-effects, especially during long-term treatment (34), alternative means of treating transplant rejection and immuninflammatory diseases are much needed.

Inhibition of interleukin 1 and interleukin 6 functions by sodium fusidate (fusidin)

Fusidic acid is a tetracyclic triterpenoic acid with a steroid-like primary structure as described above. The drug is used clinically both in its acid form and as the sodium and diethanolamine salts as an antibiotic particularly active against Staphylococcus aureus (22). It has been indicated that fusidic acid might have an inhibitory effect on T-lymphocytes in a mouse model (23A). Fusidic acid inhibits *in vivo* and *in vitro* protein synthesis in both prokaryotic and eukaryotic cells by inhibiting the attachment of transfer RNA to the 50S part of the ribosomes (23). Fusidin has few and only trivial side-effects, even during long-term treatment.

According to the present invention it has been found that fusidin acid act as an immunosuppressive agent and that its mode of action as an immunosuppressive agent closely mimicks that of cyclosporin (see the following). Hence, the potentials of fusidin therapy should be investigated in all situations where cyclosporin treatment is advocated.

As mentioned above, some of the key mediators of the body's response to environmental factors are a family of polypeptide hormones (cytokines) produced by many cell types but most abundantly by MØ. The best characterized members of the family are IL-1α and IL-1β, TNFα and the recently described IL-6 (1,2). These cytokines participate in many types of acute and chronic reactions involving inflammation, immunological reactions and tissue injury. Generally, IL-1α/β ac-

tivates cells to cell growth (e.g. fibroblasts, keratinocytes and glial cells) and/or increased activity (e.g. prostaglandin E_2 synthesis in fibroblasts, bone resorption by osteoclasts and activation of T and B lymphocytes already triggered by specific antigens or polyclonal activators). In a few instances, however, IL-1 appears to inhibit cell function. For instance, IL-1 is a potent suppressor of pancreatic islet β-cells and thyrocytes, and of melanoma cells (2,3,8,17,24). IL-1 also induces a broad spectrum of systemic changes, including fever, slow wave sleep, and changes in blood levels of trace metals, albumin and acute-phase reactants such as fibrinogen and serum amyloid A (2,8,25). IL-1α/β are therefore considered of central importance in many if not all adaptive changes of the organism in response to various exogenous and, possibly, endogenous stimuli.

Recently, IL-1α/β have been recognized to share many activities with another cytokine, IL-6 (3,7). Despite this, IL-6 and IL-1α/β do not show any sequence homology, and the cytokines act on different receptors. IL-6 has been shown to be identical with B cell-stimulating factor 2 (7) and a factor necessary for the growth of plasmacytomas and B cell hybridomas (26-28).

Unregulated production of IL-1α/β and IL-6 may be pathogenetically involved in the manifestation of several human diseases as described above, and the molecules have many biological effects which qualify them as important in the pathology leading to rheumatic diseases, some endocrine disorders, and various skin diseases (see Table 1).

Drugs that interfere with functions of IL-1α/β and IL-6 are therefore of great interest in the management of many human diseases.

As described in the examples, it has been observed that sodium fusidate (fusidin) inhibited various IL-1α/β functions *in vitro* when administered in therapeutic and non-toxic concentrations and thus prevented the lymphocyte costimulatory activities of these human cytokines. The drug was also found to interfere with the hybridoma growth-promoting activity of IL-6. Furthermore, the drug prevented

"non-immunological" functions of the cytokines, including the toxic effect of IL-1α/β on insulin production by pancreatic β-cells.

The above described functional pattern of activity is strikingly similar to that of cyclosporin, a drug used extensively to prevent transplant rejection and in the management of several immunoinflammatory disorders. Thus, it is contemplated that interference with the immunoactivating signal delivered by IL-1α/β and, possibly, IL-6 before and around transfer of a graft by fusidic acid treatment will induce specific and lasting paralysis of those host lymphocytes otherwise programmed to respond to the graft. Such treatment would spare all other clones of lymphocytes in the recipient, and life-long immunosuppressive therapy might no longer be needed. Treatment with fusidic acid or derivatives thereof is contemplated to be beneficial, according to the present invention in both the life-threatening situations dominated by DIC and in other more subtle vascular diseases (e.g. arteriosclerosis). Also, a dysregulated production and/or function of IL-6 may be a key event in the pathogenesis of at least some lymphoid proliferative diseases. Drugs that interfere with the function of the cytokine such as fusidic acid or derivatives thereof are therefore contemplated to be clinically useful. It is also contemplated that interference with the functions of IL-1α/β and IL-6 with fusidin or fusidic acid derivatives may be of therapeutic importance in several types of arthritic and degenerative joint diseases.

Use of fusidic acid according to the present invention

In accordance with the present invention it has been found that fusidic acid and derivatives thereof are useful for preventing effects of cytokines known to be pathogenetically involved in the previous described pathological conditions.

In accordance with the present invention the term "fusidic acid or a derivative thereof" comprises any pharmaceutically active and acceptable compound being identical or structurally similar to fusidic acid and exhibiting relevant biological actions similar to those of fusidic acid, including derivatives of fusidic acid,

especially pharmaceutically acceptable salts, esters and solvates as well as conjugates of fusidic acid or of the fusidic acid derivatives.

Suitable salts are salts with pharmaceutically acceptable bases, such as alkaline earth metal salts, alkali metal salts (especially sodium salts), ammonium salts or amine salts (especially the diethanolamine salts).

Suitable esters are easily hydrolysable esters, e.g. fusidic acid acetoxymethyl ester.

Suitable solvates are e.g. hydrates, in particular hemihydrates.

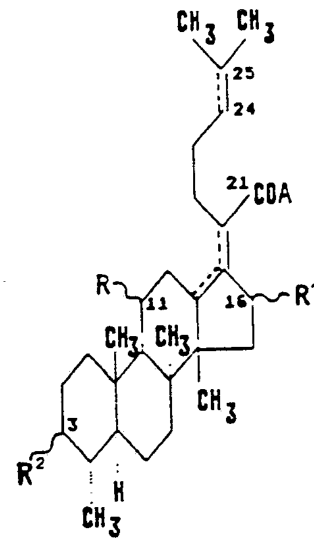
Suitable conjugates are especially conjugates with taurine or glycine.

"Derivatives of fusidic acid" comprise e.g. the following compounds:

3-dehydrofusidic acid;
3,11-didehydrofusidic acid;
24,25-dihydrofusidic acid;
17,20-24,25-tetrahydrofusidic acid;
17,20-24,25-tetrahydrofusidic acid and their corresponding 3-acetates (especially conjugated with glycine or taurine);
3-O-acetyl-16-epideacetylfusidic acid.

Also included in the present invention is the use of the physiologically acceptable derivatives, including functional derivatives, which are disclosed, as well as their preparation, in the patents and the references listed above in the section "Background of the invention", e.g. the derivatives disclosed in U.S. Patent No. 4,004,004. Some of these derivatives are insufficient as antibacterial agents - a feature which may be beneficial in the use according to the present invention.

Of particular interest in connection with the present invention are compounds having the general formula II:



II

wherein the dashed lines indicate the presence of either single or double bonds between the atoms in question, with the proviso that one of the bonds C(13-C(17) and C(17)-C(20) is a double bond,

A is selected from the group consisting of OH, OR', and NHR",
wherein R' is selected from the group consisting of
C_1-C_4-alkyl,
aralkyl, such as phenyl-C_1-C_4-alkyl, and
aryl, such as phenyl or o-, m- or p-tolyl, and
R" is CH_2COOH or $CH_2CH_2SO_3H$,

R is OH or a keto oxygen (=O), whereby, when R is OH, the C(11)-R bond is preferably in the α configuration with respect to the C(19) methyl group,

R^1 is hydrogen, halogen or a nitrile group, or R^1 is selected from the group consisting of

-OR^3, -NHR^3 and -SR^3, in which R^3 is hydrogen or is an organic group such as acyl, in particular C_1-C_4-alkylcarbonyl, alkyl, in particular C_1-C_4-alkyl, aralkyl,

such as phenyl-C_1-C_4-alkyl, or aryl, such as phenyl or o-, m- or p-tolyl,

R^2 is OH C_1-C_4-alkylsulfonyloxy or a keto oxygen (=O), whereby, when R^2 is OH, the C(3)-R^2 bond is preferably in the α configuration with respect to the C(19) methyl group, or R^2 is hydrogen or halogen or a nitrile group or a group

-OR^3, -NHR^3 and -SR^3, in which R^3 is hydrogen or is an organic group such as acyl, in particular C_1-C_4-alkylcarbonyl, alkyl, in particular C_1-C_4-alkyl, aralkyl, such as phenyl-C_1-C_4-alkyl, or aryl, such as phenyl or o-, m- or p-tolyl,

and salts thereof.

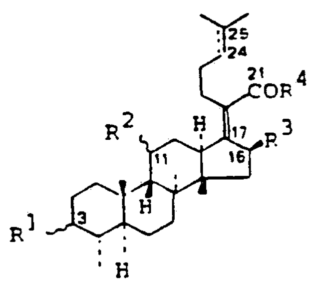
Examples of C_1-C_4-alkyl are as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sek.butyl, and tert. butyl. C_1-C_4-alkyl and the other groups mentioned above may optionally be substituted where appropriate, such as disclosed in the patents and other literature referred to in the above section "Background of the invention"

Among the compounds of the general formula II, preferred compounds of the general formula II are, e.g., compounds wherein A is OH, a double bond is present in the position C(17)-C(20) and in the position C(24)-C(25), the hydrogen atom at C(9) is in the β configuration with respect to the C(19) methyl group, R is a hydroxy group in the α configuration with respect to the C(19) methyl group, R^1 is a C_1-C_4-alkylcarbonyloxy group such as an acetoxy group or a C_1-C_4-alkylcarbonylthio group, an arylcarbonylthio group such as a phenylcarbonylthio group, or a C_1-C_4-alkoxy group such as a methoxy or ethoxy group, and R^2 is a nitrile group, a C_1-C_4-alkylsulfonyloxy group, or, in particular, a hydroxy group in the α configuration with respect to the C(19) methyl group, in particular fusidic acid and fusidic acid salts such as the sodium salt (fusidin).

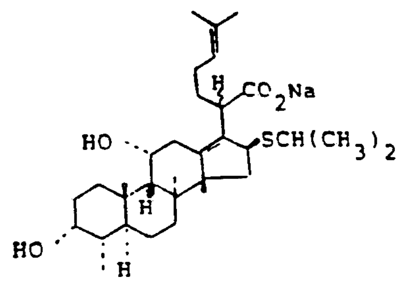
Specific examples of interesting derivatives are the derivates termed

VD 1177, 1178, 1303, 1360, 1362, 1460, 1546 and PR 1089 described in table 2 below.

TABLE 2



A



B

Code No.	Structure	Substituents				References Δ		
		R^1	R^2	R^3	R^4	Cpd. No.	Table	Page
VD 1177 Na	A, s*	α-OH	α-OH	$OCOCH_3$	$NHCH_2CH_2SO_3Na$	XVIb**	III	107,108
VD 1178 Na	A, s	α-OH	α-OH	$OCOCH_3$	$NHCH_2CO_2Na$	XVIa**	III	107,108
VD 1303 Na	A, d*	α-OH	α-OH	OCH_2CH_3	ONa	XLI	VII	120,122
VD 1360 Na	A, d	β-OSO_2CH_3	α-OH	$OCOCH_3$	ONa	LXVIc	XIII	138,139
VD 1362 Na	A, d	α-N_3	α-OH	$OCOCH_3$	ONa	LXXVI	XIV	139,140
VD 1460 Na	A, d	α-OH	α-OH	$SCOCH_3$	ONa	XLa	VI	118,119
VD 1546 Na	A, s	α-OH	α-OH	$SCOC_6H_5$	ONa	XLb**	VI	118,119
PR 1089 Na	B					LIV	X	129,130

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* s = 24,25-single bond, d = 24,25-double bond

** Structure as indicated, but 24,25-single bond

The following definitions are employed in the present text:

"Cytokine" is a general term for a proteinaceous mediator released primarily but not exclusively by a cell population of the immune system as a response to a specific stimulating agent, e.g. a specific antigen or an alloantigen; or a nonspecific, polyclonal activator, e.g. an endotoxin or other cell wall components of a gram-negative bacteria;

"lymphokine" is a general term for a proteinaceous mediator released by sensitized lymphocytes as a response to a stimulating agent, e.g. a specific antigen or an alloantigen; or by a lymphocyte challenged by a polyclonal activator, e.g. an endotoxin or other cell wall component of a gram-negative bacteria;

"interleukin" is a general term for a proteinaceous mediator released primarily but not exclusively by a macrophage, T, B or NK cell as a response to a stimulating agent, e.g. a specific antigen or an alloantigen or by a cell challenged by a polyclonal activator, e.g. an endotoxin or other cell wall component of a gram-negative bacteria;

"monokine" is a general term for a proteinaceous mediator released by a mononuclear phagocyte (e.g. a monocyte or a macrophage or a Kupffer cell (liver) or a Langerhans' cell (skin)) as a response to any stimulating agent;

"interferon" is a general term for a proteinaceous antiviral and/or monocyte/macrophage activating factor released by all cells as response to a virus or an interferon-inducer such as a polynucleotide; in particular cells of the immune system as a response to a specific stimulating agent, e.g. a specific antigen or an alloantigen, or a nonspecific, polyclonal activator, e.g. an endotoxin or other cell wall components of a gram-negative bacteria;

"colony-stimulating factor" is a general term for a proteinaceous, haematopoietic colony-stimulating mediator released primarily but not exclusively by a cell population of the immune system as a response to a specific stimulating agent, e.g. a specific antigen or an al-

loantigen; or a nonspecific, polyclonal activator, e.g. an endotoxin or other cell wall components of a gram-negative bacteria.

One aspect of the invention is the use of fusidic acid or a functional derivative thereof for the manufacture of a pharmaceutical composition for substantially inhibiting a biological effect in a human related to a cytokine, such as a lymphokine, interleukin, monokine, interferon or colony-stimulating factor, for the prophylaxis or treatment of a condition related to a disturbance of a cytokine system such as the lymphokine, interleukin, monokine, interferon or colony-stimulating factor system. As used herein the term "pharmaceutical composition" comprises any composition suitable for human use.

The invention particularly relates to the use of fusidic acid or a functional derivative thereof

- for substantially *inhibiting* a biological effect in a human related to a cytokine for the prophylaxis or treatment of a condition related to a disturbance of a cytokine system; and/or

- for substantially *inhibiting* a biological effect in a human related to a lymphokine for the prophylaxis or treatment of a condition related to a disturbance of a lymphokine system; and/or

- for substantially *inhibiting* a biological effect in a human related to an interleukin for the prophylaxis or treatment of a condition related to a disturbance of a interleukin system; and/or

- for substantially *inhibiting* a biological effect in a human related to a monokine for the prophylaxis or treatment of a condition related to a disturbance of a monokine system, and/or

- for substantially *inhibiting* a biological effect in a human related to an interferon for the prophylaxis or treatment of a condition related to a disturbance of a interferon system; and/or

- for substantially *inhibiting* a biological effect in a human related to a colony-stimulating factor for the prophylaxis or treatment of a condition related to a disturbance of a colony-stimulating factor system.

As used herein "substantially inhibiting" comprises a therapeutically relevant prevention of the harmful biological actions of said mediator, said prevention being based on fusidic acid or derivatives thereof or combinations with other treatments/drugs exerting a form of antagonistic effect on the actions of the mediator in question.

Said conditions have been described above, and the conditions described comprise diseases occuring in humans which diseases demand therapeutical intervention as well as conditions in which a suppression of a cytokine system is desirable such as in connection with transplantation or eye surgery. As has been described in detail above, clinically important interleukins in this context are in particular interleukin 1 (α or β), and interleukin 6.

In one of its broadest aspects the invention further relates to the use of fusidic acid or its derivatives as a substantially selective immunosuppressive drug and/or as a drug the action of which is via an antagonistic effect on the action of one or more cytokines.

Whenever systemic treatments are employed, the administration may be orally, rectally and/or parenterally, the administration being dependent on the patient's age and weight and the particular condition being treated as well as the severity of the disease.

The active ingredient or combinations thereof may be contained in any appropriate amount in a composition, and are generally contained in an amount of 1-95% by weight, based on the total weight of the preparation. The composition may be in any appropriate unit dosage form.

Parenteral administration may comprise suitable injection, e.g. intravenous, intramuscular, intraarticular, intraocular or retroocu-

lar injection, infusion or implantation of e.g. suitable delivery devices.

Formulations for parenteral use may be presented in unit dose forms, e.g. ampoules, or in multi-dose containers with an added suitable preservative. The composition may be in form of a solution, a suspension, an emulsion or a delivery device for implantation or may be presented as a dry powder to be reconstituted with water or another suitable solvent before use. Apart from the active drug substance the compositions may comprise suitable pharmaceutically acceptable carriers and/or exipients. Furthermore the composition may, in addition, conveniently comprise suspending, stabilizing, pH-adjusting agents and/or dispersing agents.

Compositions for oral or rectal use may be formulated according to conventional pharmaceutical practice and may be in the form of tablets, capsules, pills, powders, ampoules, granulates, dragées, pastes, gels, suppositories or enemas; or liquid formulations such as suspensions (oily or aqueous), solutions, elixirs, emulsions or drenches and the like.

The auxiliary additives of the pharmaceutical compositions may be any conventional pharmaceutical additives and carriers:

Binding agents such as cellulose derivatives, starch, gelatin and polyvinylpyrrolidine; fillers such as sugar, mannitol, lactose, microcrystalline cellulose, potato starch, calcium phosphate; suitable absorption promotors; disintegrants such as potato starch, alginic acid; lubricants such as magnesium stearate, stearic acid, talc; emulsifying agents such as lecithin, sorbitan monooleate; wetting agents such as lecithin, polyoxyethylene esters; or buffering agents such as acetate, citrate, phosphate.

The solid compositions may by means of specially adapted coating techniques be provided with a coating adapted to protect the composition from unwanted chemical changes prior to the release of the active compound. The coating may be adapted to release the active

compound in a predetermined pattern e.g. in order to achieve a controlled release formulation.

The dosage of fusidic acid or its derivatives depend on the administration method, the disease, the severity of the disease and of the weight and age of the patient. For compositions adapted for oral administration, the dosage is often 3.5 mg-1 g administered 2-6 times daily or for instance 0.5 g x 5 daily or in certain cases 1 g every second or third day. Thus, oral administration may comprise ranges from approximately 1 mg to 75 mg per kg body weight per day or in appropriate cases 0.1 mg to 20 mg per kg body weight per day. A preferred dosage regime is normally 0.5 g 3 times daily. In certain cases, e.g. in the treatment of graft-versus-host disease the dosage is normally doubled. When fusidic acid or a derivative thereof is administered rectally, a somewhat higher amount such as from about 1 mg to about 100 mg per kg body weight per day is usually preferred.

For parenteral administration, a dosis of about 0.1 mg to about 50 mg per kg body weight per day, most preferably in an amount from about 1 mg to about 20 mg per kg body weight per day is convenient. When administering fusidic acid intraarticularly, an amount from about 0.1 mg to 20 mg per kg body weight per day is usually preferable. For parenteral administration, an aqueous solution of e.g. 0.5-2% or more of the active ingredient may be employed.

When fusidic acid or a derivative thereof is administered to the eye, an amount from about 0.1 mg to about 50 mg per kg body weight per day is usually preferred.

In every case, the dosage should be carefully adapted so as to imply the specific action in the cytokine system, i.e. attainment of optimal total dosages, dosage forms and dosage frequency. In certain cases it is relevant to administer a relatively high unit dosis, a so-called "booster dosis".

Pharmaceutical compositions for topical use such as compositions suitable for application to the skin according to the present invention are suitably creams, gels, ointments, lotions, liniments,

suspensions, solutions, pastes, sticks, sprays, soaps, shampoos, powders, suppositories and enemas. The topical administration should be onto or close to the pathological changes in the body. The compositions may be any suitable medicated mass adapted for direct application or for introduction into the relevant orifice of the body, e.g. the rectal, urethral or vaginal orifice. The compositions may simply be applied directly onto the diseased part, e.g. the skin or mucosa. Other relevant formulation adaptations for application to the eye may e.g. be eye lotions, eye ointments, eye-drops or drug delivery systems adapted for administration to the eye such as compositions suitable for implantation administration. In certain cases it may be applied by means of special medical devices such as dressings including occlusive dressings, or alternatively plasters, pads, sponges, strips, or other forms of suitable flexible pieces of material.

Formulations for topical administration may comprise pharmaceutically acceptable carriers and/or exipients such as ointment bases (e.g. paraffin, polyethylene glycols, Tween®, Span® , vegetable oils), suspending or emulsifying agents (e.g. lecithin, sorbitan monooleate, gelatin, methyl cellulose, gum acacia, sorbitan monooleate derivatives), gelforming agents (e.g. Carbopol, alginates, gelatin), preservatives (e.g. methyl or propyl p-hydroxybenzoates, benzalkonium chloride), antioxidants (e.g. tocopherol, ascorbic acid, butylated hydroxy anisol), humectants (e.g. glycerin, propylene glycol, urea), and perfumes and skin protective agents.

Formulations for topical administration to the eye may comprise pharmaceutically inert vehicles or be in form of a suitable carrier system. Pharmaceutically acceptable carriers and/or exipients for the preparation of eyedrops include for example buffering agents such as boric acid or borates, pH adjusting agents to obtain optimal stability or solubility of the actuve drug substance, agents to adjust the tonicity such as sodium chloride or borates, viscosity altering agents such as hydroxypropyl cellulose, methylcellulose, polyvinylpyrrolidone, polyvinyl alcohols or polyacrylamide, oily vehicles such as vehicles comprising arachis oil, castor and mineral oils. Eyedrops presented as emulsions or suspensions may furthermore comprise stabi-

lizing, dispersing, wetting, emulsifying and/or suspending agents. Eye lotions and eye ointments may comprise pharmaceutically acceptable carriers and/or exipients such as used in an eyedrop composition or in other relevant topical composition, e.g. ointments, creams, lotions and the like.

An example of a general method for preparing aqueous eyedrops comprising an active drug substance is to dissolve the compound (preferably as the soluble sodium salt) in sterile water in a given concentration, optionally adjust the pH to a suitable value with a suitable buffer or with hydrochloric acid or sodium hydroxide, optionally add a preservative like phenethanol or chlorobutanol, optionally add a viscous altering excipient like methylcellulose, and sterilize the final solution by e.g. autoclavation by use of one of the generally accepted cycles or by membrane filtration.

The topical compositions for use according to the present invention comprise e.g. 0.001-60% w/w of the active ingredient, preferably 0.1-20%, especially 0.5-10% or 1-5%.

According to the invention the compositions may be applied several times a day, e.g. 1-5 times a day, depending on the condition in question and the severity thereof and furthermore on the absorption conditions on the site in question.

In each case, the particular dosis will depend on obtaining an appropriate absorption of the fusidic acid or derivative thereof in a sufficient form and amount. In each case, said amount and form should be adapted so as to exert its specific action in the cytokine system. Hence, it is very important - e.g. when application onto the skin is performed - to ensure that the patient applies a relatively well-defined layer of the composition.

In particular, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of diabetes mellitus, in particular insulin-dependent diabetes mellitus (type 1), in particular for the prevention of progression of diabetes mellitus (type 1), especially substantially immediately after the first

diagnostic establishment of diabetes mellitus, or for prophylaxis after establishment of being in a high risk group of developing diabetes mellitus.

These uses as well as other uses and methods of the invention will preferably be noted specifically and in detail as explained herein in instructions for use provided to the physician and/or to the patient together with the compositions to be used.

During the last few years animal investigations have shown the capability of cyclosporin (a cyclic peptide metabolite of the fungi cylindrocarpon lucidum and trichoderma polysporum) to prevent diabetes, first of all in the spontaneously diabetic BB rat.
Clinical studies performed on diabetics and involving investigation of cyclosporin's effect on progression of diabetes have shown that clinical remission was obtained by means of the cyclosporin therapy; this seemed to be due to improvement of the functional β-cell mass. The remission rate was greater in diabetic patients who entered after less than six weeks with symptoms and two weeks of insulin therapy, than in those who entered later. The findings demonstrate that an acute process affecting the β-cells can be modulated by initiation of the cyclosporin immunosuppressive therapy within a critically brief time interval after onset of overt disease, and that continued treatment can maintain partial or complete remission through a period of at least one year.

As used in the present specification and claims, the term "substantially immediately after" comprises a period after the first diagnostic establishment of the disease or a high risk of developing the disease within a limited number of months or years which period should be as short as possible, so as to enable intervention with the progression of irreversible changes in the pathological body tissues, said invention being obtained by means of the use according to the invention of fusidic acid or derivatives thereof.

The administration of cyclosporin increased the remission rate in diabetes mellitus (type 1) of recent onset, and enhanced and preserved β-cell function through the first year after diagnosis. It is

inferred that these effects were induced by virtue of the immunosuppressive action of cyclosporin. Thus the findings strongly support the hypothesis that an immune process is involved in β-cell damage in the human disease.

The effect of duration of diabetes on the early outcome suggests that although the immune processes affecting β-cells may be chronic and precede diagnosis through a long period of time, the development of overt disease is associated with an acceleration of events.

From e.g. the above-described experiments it is inferred that the potentiality for recovery of insulin secretion is greater than formerly believed, and that by administration of fusidic acid or a derivative thereof, the secretory capacity can be maintained in the normal range through one year or more of immunosuppression in many patients.

Thus, fusidic acid and derivatives thereof may be used according to the present invention for the prevention of progression of irreversible pathological processes in the pancreatic tissues which otherwise lead to diabetes mellitus (type 1). The therapy of the invention is preferably instituted as soon as possible, i.e. very shortly after the onset of diabetes or, preferably, immediately after diagnosis of pathological processes in the islets of pancreas (e.g. before clinical manifestation of diabetes mellitus (type 1)). The therapy of the invention may also apply to certain identified high risk groups the identification being e.g. obtained by means of a screening program employing markers relatively specific for pathological changes seen in diabetes mellitus and/or preliminary stages thereof, especially markers of ongoing pathological changes in predisposed individuals (e.g. in HLA-DR 3/4-positive individuals). A person is contemplated to be in a high risk group of developing diabetes mellitus (type 1) if his risk is about 10-100%, such as about 50-100%.

In particular, the present invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of diabetes mellitus (type 1) for oral or rectal use.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the prophylaxis or treatment of conditions related to the function of the thyroid gland, in particular hyper- or hypofunctioning of said gland, e.g. thyroiditis (acute, subacute or chronic), including Hashimoto's disease (lymphocytic thyroiditis; lymphoid thyroiditis), Riedel's thyroiditis (chronic fibrous thyroiditis), de Quervain's thyroiditis (subacute granulomatous thyroiditis), subacute lymphocytic thyroiditis, Graves' disease, Graves' subacute thyroiditis and Graves' ophthalmopathy.

For a plurality of diseases e.g. the conditions related to the function of the thyroid gland, the inflammatory diseases of the gut, arthritis urica, multiple sclerosis, B and T cell lymphoma such as multiple myeloma treatment with fusidic acid or derivatives thereof can be beneficial to the patient also in a period of inapparant disease, in the way that the treatment with fusidic acid or derivatives thereof can prevent or postpone a relapse of the condition in question. By the term "treatment" is therefore generally also meant prophylactic treatment designated to prevent or postpone a relapse of the disease.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the prophylaxis or treatment of a condition related to hypofunction of the adrenal glands, in particular Addison's disease or furthermore Simmonds' panhypopituitarism.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the treatment of endogenous uveitis. By the term "endogenous uveitis" is meant e.g. non-infectious uveitis, such as non-infectious uveitis anterior and uveitis posterior. The present invention relates in particular to the manufacture of a composition suitable for treatment of the eye, such as an eye drop composition, an eye ointment composition, an eye lotion composition or an injectable composition for intraocular or retroocular injection, or an oral composition.

In yet another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the prevention of the inflammation after eye surgery such as cataract operation or laser surgery. This treatment, as well as treatment in other conditions, can in some cases be prophylactic in the way the the goal of the treatment is to diminish or avoid an inflammation not to actually treat the condition. The present invention relates in particular to a composition suitable for treatment of the eye, such as an eye drop composition, an eye ointment composition, an eye lotion composition or an injectable composition for intraocular or retroocular injection or an oral composition.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for treatment of progression of arthritis such as rheumatoid arthritis including juvenile rheumatoid arthritis, psoriatic arthritis or Reiter arthritis substantially immediately after the first diagnostic establishment of arthritis. The therapy of the invention is preferably instituted as soon as possible, i.e. very shortly after the onset of arthritis or, preferably, immediately after diagnosis of pathological processes (e.g. before clinical manifestations). The therapy of the invention may also apply to certain identified high risk groups the identification being e.g. obtained by means of a screening program employing markers relatively specific for arthritic changes and/or preliminary stages of arthritis, especially markers of ongoing pathological changes in predisposed individuals, in particular individuals having specific HLA tissue types. The invention relates in particular to the manufacture of a composition for oral use or for use as parenteral or intraarticular injections.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the prophylaxis or treatment of osteoarthrosis.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the prophylaxis or treatment of arthritis urica (gout).

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of a condition related to transplant rejection, such as a graft-versus-host reaction, or

any other conditions related to e.g. marrow, cornea or skin transplantation. The treatment often requires higher doses of fusidic acid than usual and is often instituted as a prophylactic treatment to avoid transplant rejection or graft-versus-host disease. In particular, the present invention relates to a such composition for oral or parenteral administration.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the prophylaxis or treatment of a condition related to cornea transplantation, in particular to a composition suitable for treatment of the eye, such as an eye drop composition, an eye ointment composition, an eye lotion composition or an injectable composition for intraocular or retroocular injection, or an oral composition.

In yet another aspect, the invention relates to the use of fusidic acid or a derivative thereof for the treatment of Crohn's disease or ulcerative colitis, especially for the prevention of relapse or progression of Crohn's disease or ulcerative colitis, or for the treatment of perniceous anemia or celiac disease, in particular to the manufacture of an oral or rectal composition, or a composition for parenteral administration.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of contact dermatitis or allergic/atopic dermatitis, or for treatment of pemphigus vulgaris or pemphigoid, in particular to the manufacture of a composition for oral administration, or for topical treatment of contact dermatitis, in particular for topical administration to the skin.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for treatment or prevention of relapse of a demyelinating disease, in particular multiple sclerosis, or for

treatment or prevention of relapse of sarcoidosis Boeck, Sjögren's syndrome, Reiter's syndrome, erythema nodosum, scleroderma or Bechet's disease.

In yet another aspect, the invention relates to the use of fusidic acid or a derivative thereof for treatment or prevention of relapse of polymyositis polymyalgia rheumatica, myocarditis or systemic lupus erythematosis, in particular to a the manufacture of a composition for oral or parenteral administration.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for treatment or prevention of relapse of a condition related to vasculitis phenomena, e.g. polyarteritis nodosa, Wegener's granulomatosis, or giant-cell arteritis.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for treatment or prevention of relapse of primary biliary cirrhosis or chronic active hepatitis.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for treatment of a neoplastic disease, such as
aplastic anemia or idiopathic thrombocytopenic purpura, or for treatment or prevention of relapse of a neoplastic disorder of the lymphoid tissue, e.g. B cell lymphoma or multiple myeloma, or a T lymphocyte proliferative disorder, e.g. mycosis fungoides or Sézary syndrome. The present invention relates in particular to a such composition for oral or parenteral administration.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of septic shock caused by gram-negative bacteria. Fusidic acid has been used for treatment of infections caused by *gram-positive* bacteria such as Staphylococcus aureus, but prior to the present invention it was not known that fusidic acid could be used for prophylaxis or treatment of septic shock which is caused by *gram-negative* bacteria. The present invention relates in particular to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of septic shock

caused by gram-negative bacteria for oral or parenteral administration.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of disseminated intravascular coagulation.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis of arteriosclerosis.

In another aspect, the invention relates to the use of fusidic acid or a derivative thereof for prophylaxis or treatment of a condition acute and chronic periodontal diseases, in particular periodontitis and periodontosis, in particular by means of periodontal injections.

In yet another aspect, the invention relates to the use of fusidic acid or a derivative thereof for use as an immunosuppressive drug, and the use in combination with other relevant drugs, such as cyclosporin or a derivative thereof.

In a further aspect, the invention relates to the use of fusidic acid or a derivative thereof together with a cyclosporin (e.g. Cyclosporin A and G) or a derivative thereof. Such combination therapy is designed so as to reduce the serious adverse effects of the cyclosporin, in particular the nephrotoxic effects, and at the same time utilizing the immunomodulating effects of the cyclosporin together with the effects of fusidic acid or its derivatives. Relevant dosage intervals may e.g. be:

Cyclosporin A (or another cyclosporin of equivalent potency):

- Systemic administration: 1-10 mg/kg body weight;
- Topical administration: 1-20 mg/ml (ointments, solutions, eye drops etc., cf. above).

Also provided by the present invention is a pharmaceutical composition comprising fusidic acid or a derivative thereof together with a cyclosporin (e.g. Cyclosporin A and G) or a derivative thereof.

In a further aspect, the invention relates to the use of fusidic acid or a derivative thereof together with a non-steroid antiinflammatory drug (NSAID) (e.g. indometacin and acetyl salicylic acid) or an analogue and/or a glucocorticoid (e.g. hydrocortisone). Such combination therapies are designed so as to reduce the serious adverse effects, and at the same time utilizing the effects of said drugs together with the effects of fusidic acid or its derivatives. Relevant dosage intervals may e.g. be:

Acetyl salicylic acid (or another NSAID of equivalent potency):

- Systemic administration: 5-100 mg/kg body weight;
- Topical administration: 10-100 mg/ml (ointments, solutions, eye drops etc., cf. above).

Hydrocortisone or another glucocorticoid drug of equivalent potency:

- Systemic administration: 1-20 mg/kg body weight;
- Topical administration: 1-20 mg/ml (ointments, solutions, eye drops etc, cf. above).

In yet a further aspect, the invention relates to use of a pharmaceutical composition comprising fusidic acid or a derivative together with a non-steroid antiinflammatory drug (e.g. indometacin and acetyl salicylic acid), and/or glucocorticoid (e.g. hydrocortisone).

In another aspect, the invention relates to the use of fusidic acid or a functional derivate thereof together with FK-506 or a functional derivative thereof. Such combination therapy is designed so as to reduce the serious adverse effects of FK-506, and at the same time utilizing the immunomodulating effects of the FK-506 together with the effects of fusidic acid or its derivatives.

In every case, the dosage of the additional drug or drugs described above should be carefully adapted so as to imply the specific action in the cytokine system, i.e. attainment of optimal total dosages, dosage forms and dosage frequency. In certain cases it is relevant to administer a relatively high unit dosage, a so-called "booster dosis".

EXAMPLES

IN VITRO EXPERIMENTS

MATERIALS AND METHODS

Fusidin, fusidic acid derivatives, and cyclosporin

The water soluble sodium salts of fusidic acid and fusidic acid derivatives as well as the fucithalmic eyedrops and the fusidin tablets were gifts from Leo Pharmaceutical Products (Ballerup, Denmark). Except for 2 derivatives (see below), the drugs were dissolved in 0.1 M phosphate buffered saline, pH 7.4 (PBS) at a concentration of 1 mg/ml and stored at 4°C.

The fusidic acid derivatives VD1546Na and PR1089Na were dissolved at 100 mg/ml in 98% ethanol and stored at 4°C. Before each experiment,

the compounds were further diluted in culture medium RPMI 1640 (Roswell Park Memorial Institute) with 25 mM hepes-buffer (NordVacc, Stockholm, Sweden) supplemented with penicillin (500 IU/ml), streptomycin (500 μg/ml), L-glutamine (2 mM), and 1 - 10% of a heat-inactivated human serum pool (NHS). The final concentrations of ethanol were always less than 0.5 o/oo (v/v), and these concentrations did not affect the production or function of the cytokines.

Cyclosporin was kindly provided by Sandoz (Basel, Switzerland). The powder was dissolved in ethanol (50 mg/ml) and diluted to 20 μg/ml in sterile saline. This stock solution was stored at -20°C until use. A similar dilution of ethanol was stored and used in parallel experiments.

Human cytokines

rIL-1α was a gift from T. Tsuboi (Dainippon, Osaka, Japan). rIL-1β was from Cistron Biotechnology (Pine Brook, NJ, USA). Purified native human IL-6 and rIL-6 used for pancreatic β-cell experiments were donated by J. van Damme (University of Leuven, Leuven, Belgium)(26) and L.A. Aarden (University of Amsterdam, Amsterdam, Holland)(27). rIL-6 used for the other studies was a gift from T. Hirano (Osaka University, Osaka, Japan)(28). rTNFα was a gift from G.R. Adolf (Ernst Boehringer Institute, Vienna, Austria). rIL-2 was from Boehringer (Mannheim, FRG). The activities of the cytokines were ascertained by bioassays (see below). The cytokines were calibrated with WHO interim standard preparations of the human cytokines (NBSB, London, U.K.). The endotoxin contents of the cytokine preparations did not exceed 1 pg/1,000 U measured by a chromogenic Limulus Amebocyte Lysate assay (Struers, Rødovre, Denmark).

Production of cytokines

Human mononuclear cells (MNC) were isolated from the blood of healthy, unmedicated adult donors by gradient centrifugation of heparinized venous blood on Ficoll-Hypaque (Lymphoprep, Nyegaard, Oslo, Norway), as previously described (20,29-32).

Cells, $2x10^6$/ml, were incubated in culutre medium RPMI 1640 and pulsed for 1h at 37°C with 2 μg/ml of *E. coli*-endotoxin 055:B5 (LPS) extracted by the hot-phenol-water method (Mallinkrodt Inc., St. Louis, MO, USA) plus 10 μg/ml of phytohemagglutinin-P (PHA)(Difco, Detroit, MI, USA). The cells were then washed two times with Hanks' balanced salt solution (HBSS) and resuspended in the same culture medium containing 2 μg/ml of LPS but not PHA. In some experiments, the production of cytokines were carried out in the presence of 1 μg/ml of indomethacin (Sigma, St. Louis, MO, USA). After 20 h of culture, the supernatants were isolated and dialyzed for minimum 2 days at 4°C against HBSS and, subsequently, RPMI/Hepes buffer (20). The cells were collected for viability studies using trypan blue exclusion. In parallel experiments, 20 μg/ml of sodium-11β-^{3}H-fusidate, specific activity 93 μCi/mg, (Leo Pharmaceutical Company) was added at the initiation of the cultures using 0-10% (v/v) NHS. After dialysis, the supernatants were tested for residual ^{3}H fusidic acid by scintillation counting.

T cell growth induced by IL-2

Supernatant IL-2 activity and the effect of fusidin on IL-2 activity *in vitro* were measured using IL-2-dependent CTLL-2 cells (American Type Culture Collection, Rockville, MD, USA) cultured for 20 h at 37°C and pulsed with ^{3}H-thymidine for the last 3 h of culture (31). All experiments were carried out in triplicate.

ELISAs for TNFα and IFNγ

The presence of TNFα was measured by a slight modification of a previously described sandwich ELISA, using high-titered, monospecific rabbit antisera to human rTNFα (30). The ELISA utilizes the biotin/avidin system, and the detection limit is 1 pg/100 μl. The assay is not influenced by serum, and there is no cross reaction with other cytokines, including TNFβ (=lymphotoxin). The recovery of TNFα was always above 85% and the coefficient of variation within and between assays were less than 10%.

IFNγ was measured by a sandwich ELISA purchased from Holland Biotechnology (Leiden, Holland). The ELISA utilizes two different monoclonal antibodies to IFNγ and the biotin/antibiotin-alkaline phosphatase method. The sensitivity of this assay was 15 pg per 150 μl, and the coefficient of variation was less than 10%. In the initial experiments, human IFNγ was tested by immunoradiometric assay (IRMA) using two monoclonal antihuman IFNγ antibodies (Centocor, Malvern, PA, USA). The sensitivity of this assay is approximately 0.1 IU/ml.

T cell proliferation induced by IL-1α/β

Total IL-1 activities in the supernatants were measured by 3 bioassays using 1) murine T cells (thymocytes), 2) human enriched blood T lymphocytes (both assays also detect IL-6), or 3) mouse lymphoma cells (EL 4) (do not detect IL-6).

The thymocyte co-stimulatory assay for IL-1 was carried out as previously described (29). Briefly, thymocytes from endotoxin-resistant C3H/SSI, female mice, 6-8 weeks of age (Bomholtgaard, Ry, Denmark) were grown in triplicate in culture medium supplemented with 10% (v/v) NHS and $5 x 10^{-5}$M mercaptoethanol in 96-wells microtiter plates (Nunc, Roskilde, Denmark), 10^6 cells/ 200 μl/well. Two-fold dilutions of test material and 10 μg/ml of PHA were added at the initiation of the cultures. After 48 h, the cells were pulsed with ^{3}H-thymidine (0.5 μCi/well) for 24 h, harvested on paper filters and counted by liquid scintillation.

Purified human T-lymphocytes were prepared from MNC as described (29A). In brief, MNC were depleted of adherent cells by incubation for 1 h at 37°C on plastic tissue culture dishes (Becton Dickinson, Oxnard, CA, U.S.A.). Nonadherent cells were removed and passed through a nylon wool column, and the resulting cell population consisted of more than 80% T cells, 15% B cells, and less than 1% MO.

The EL 4 assay was carried out as described (29). It is based on the production of IL-2 from the murine thymoma cell line (EL 4) in the presence of 1.2×10^{-7} M of the calcium ionophore, inonomycin (Sigma), and IL-1α or IL-1β. Briefly, 2×10^5 EL 4 cells/200 μl were

incubated in triplicate for 18 h with 2- fold dilutions of test material. After incubation, the supernatants were collected and tested for IL-2 activity using IL-2-dependent CTLL-2 cells (31). Calculations of activities were carried out by a computerized linear regression analysis of probit-transformed data using serial 2-fold supernatant dilutions.

Hybridoma growth induced by IL-6

IL-6 activity was determined by ^{3}H-thymidine incorporation into IL-6-dependent mouse hybridoma cells, line B 13.29 clone B9, essentially as described by Aarden et al. (27). Briefly, triplicate samples each containing 5 x 10^3 B9 cells were added serial dilutions of test material and cultured for 3 days at 37°C. ^{3}H-Thymidine was added for the last 5 h of culture, and the cells were harvested and assayed by liquid scintillation. A titration curve of a standard human rIL-6 preparation was carried out in each assay. One unit/ml of IL-6 activity was defined as the concentration of the laboratory standard giving half maximal ^{3}H-thymidine incorporation using computerized probit analysis (1 unit (=) 1 pg of human rIL-6).

Fibroblast toxicity induced by TNFα

The TNF-induced cytotoxicity was tested on L-M mouse fibroblasts as previously described (30). A titration curve of a standard human rTNFα preparation, previously calibrated with a WHO interim reference preparation, was carried out in each assay. Calculations of activities were carried out by a computerized linear regression analysis of probit-transformed data using serial 3-fold supernatant dilutions.

Human mixed lymphocyte reaction

The mixed lymphocyte cultures (MLC) were performed by adding human blood MNC, 10^5/100 µl, to the same amount of blood MNC from an unrelated healthy donor. The cells, 2x10^5 in 200 µl culture medium RPMI 1640 and 10% NHS, were then incubated at 37°C for 5 days. The incorporation of ^{3}H-thymidine, added 16 h before termination of the cultures, was determined by liquid scintillation.

Insulin production by isolated rat islets

Islets from collagenase-digests of pancreas tissues, obtained from Wistar rats (90-120 g), were isolated and precultured for 7 days, essentially as previously described (32). The islets were counted, pooled and washed two times in culture medium RPMI 1640, supplemented with penicillin (500 IU/ml) streptomycin (500 μg/ml), amphotericin B (2.5 μg/ml), L-glutamine (2 mM), 25 mmol/l Hepes buffer, 11 mmol/l glucose, and 0.5% NHS. Islets, 25/1 ml, were distributed at random in 24-well culture plates (Nunc). The cultures were performed in triplicate. After 5-7 days at 37°C in a 5% CO_2-humidified air atmosphere, 0.2 ml of culture medium were removed for insulin determination by a radioimmunoassay using rat insulin as standard.

Bone resorption induced by IL-1α

Calvaries from 5-day-old mice, injected subcutaneously 2 days before with ^{45}Ca, were removed and cultured as previously described (32). The parietal bones were cut into 4 pieces, 2 acting as controls and 2 for experiments. The isolated bone pieces were cultured in test tubes containing 1 ml of medium TC-199 (Gibco Bio-cult, Paisley, Scotland), supplemented with 5 g/l of bovine serum albumin fraction V and 40 mg/l of ampicillin.

The cultures were incubated at 37°C in 5% CO_2-humidified air atmosphere. After 48 h, each calvarie-piece was removed and decalcified for 30 min at 90°C in 1 ml of 1N HCl. Aliquots, 250 μl, from these solutions, and from the medium, were then assessed by standard liquid scintillation counting. The release of calcium was calculated as a percentage of the total amount of ^{45}Ca found in the medium (the release of ^{45}Ca from dead bone tissue was not subtracted). The effects were expressed as a ratio between the treated and control bones.

CALCULATIONS AND STATISTICAL EVALUATIONS OF THE EFFECTS OF FUSIDIN AND FUSIDIC ACID DERIVATIVES

The cells were always precultured for 15 min with sodium fusidate (fusidin), or fusidic acid analogues, or with the solvent alone (control) before adding the human natural or recombinant cytokine preparations to be assayed. The amounts of added cytokines varied in accordance with the sensitivities of the various bioassays, but they were always chosen to ensure suboptimal effects ((=) 50% of maximum activity) on their respective target cells. To test for possible enhancements of cytokine functions, experiments were also carried out with minimally effective amounts of added cytokines ((=) 10-20% of maximum activity).

In experiments designed to test the effect of fusidin on lymphokine production by blood MNC, the lymphokine-containing supernatants were dialyzed to ensure removal of fusidin before they were measured for their cytokine contents (see above).

The inhibitory effects of fusidin or fusidic acid analogues were usually expressed as per cent inhibition of cytokine activity, according to the formula:

$$\% \text{ Inhibition} = \frac{\text{median value (in the presence of fusidin)}}{\text{median value (in medium alone)}} \times 100$$

P values are determined by the Mann-Whitney rank sum test or Wilcoxon's test for paired differences.

EXAMPLE 1

Fusidin-induced inhibition of interleukin 1 production by human MØ

The test was performed as described in "Production of cytokines" and "T-cell proliferation induced by IL-1α/β".

As shown in Fig. 2, fusidin progressively inhibited IL-1 production from LPS-PHA-activated MNC *in vitro*. The viability of the cells always exceeded 80% after 1 day in culture, and the production of another MØ-derived cytokine, TNFα, was completely unaffected (Fig. 2).

EXAMPLE 2

Fusidin-induced inhibition of lymphokine production by human T lymphocytes

The test was performed as described in "Production of Cytokines", "T cell growth induced by IL-2" and "ELISAs for TNFα and IFNγ".

As shown in Fig. 3, increased concentrations of fusidin progressively inhibited the release of IL-2 and IFNγ by LPS-PHA-activated MNC *in vitro*. Fifty % reduction in the production of these lymphokines were achieved with 5-15 μg/ml of fusidin. Under similar conditions, half maximal production of these cytokines was obtained with 15-50 ng/ml of cyclosporin. As noted above, the relatively lower concentration activity level of fusidin compared to cyclosporin is not a disadvantage, considering that toxic concentrations of cyclosporine are in the range from 100 ng/ml and above. With fusidin, on the other hand, there is no significant toxicity in concentrations up to about 200 μg/ml, so that the therapeutic index of fusidin is at least 5 times higher than that of cyclosporin. IL-2 and IFNγ are produced by the T-lymphocytes, and these cells require IL-1 as a co-stimulatory signal even in the majority of cases where polyclonal, nonspecific T-cell activators are being used (see Fig. 1 and ref. 1-4).

As indicated above, fusidin was not toxic to the cells, judged by trypan blue exclusion. The cellular viability always exceeded 80%.

To rule out the possibility of carry-over of fusidin to the assay system, especially important in the case of the bioassay of IL-2, experiments were carried out in the presence of radiolabelled fusidin. These experiments confirmed that fusidin was completely elimina-

ted from the supernatant material by the dialysis procedure, even in the presence of 10% NHS and with only 1 day of dialysis at 4°C.

To test whether fusidin acted by an increased production of prostaglandins, some of which are known to suppress the production of cytokines (4), experiments were carried out in the presence of indomethacin throughout the culture period. Indomethacin is a cyclooxygenase inhibitor and therefore blocks the production of prostaglandins. However, in 4 different experiments indomethacin did not modify the suppression of lymphokine production afforded by fusidin.

EXAMPLE 3

Fusidin-induced effects on cytokine functions

3.1.IL-1

The test was performed as described in "T cell proliferation induced by IL-1α/β".

3.1.1. Mouse thymocytes

As shown in Fig. 4, fusidin inhibited the thymocyte co-stimulatory effect of rIL-1α and rIL-1β in a dose-related manner in this conventional test for IL-1 activity. A 50% reduction in IL-1α and IL-1β activities was achieved at 1.5-5 μg/ml of fusidin. Under similar test conditions, half maximal IL-1 response required 15-50 ng/ml of cyclosporin.

Since fusidin is bound extensively although reversibly to protein, increased concentrations of serum might nevertheless interfere with the IL-1 inhibitory function of the drug. Experiments were therefore carried out with serum concentrations of 1%, 3% and 10% (v/v), respectively. As shown in Table 3, the highest serum concentration only slightly reduced the ability of the drug to interfere with IL-1-induced thymocyte activation.

TABLE 3

Effect of various serum concentrations on the ability of fusidin to block IL-1-induced thymocyte activation

Fusidin conc. (μg/ml)	% inhibition 1% serum	3% serum	10% serum
10	89	93	87
30	96	95	87

10 and 30 U of rIL-1α or rIL-1β were added to the thymocyte assay with or without fusidin. There was no difference between the two species of IL-1. The results are means of 4 different experiments, each with the use of 2 different concentrations of rIL-1α and rIL-1β.

3.1.2. Human T lymphocytes

Fig. 5 shows the effect of fusidin on the IL-1 co-stimulatory acitivity using enriched human T lymphocytes. Significant inhibition was obtained at non-toxic concentrations of fusidin, especially when PPD was used as a co-stimulator to simulate antigen-induced activation of T cells.

3.1.3. Mouse lymphoma cells (EL 4)

Because thymocytes contain a small number of macrophages and dendritic cells in addition to T lymphocytes, the ability of fusidin to inhibit IL-1α-induced activation of a mouse thymocyte cell line (EL 4) was next investigated. As shown in Fig. 6, fusidin also appeared to inhibit rIL-1α-induced proliferation of EL 4 cells. The fact that the effect of the drug was much less pronounced than in Examples 3.1.1. and 3.1.2. can most likely be attributed to induction of EL 4 cell proliferation requiring co-stimulation with a calcium ionophore, in addition to IL-1. Another possibility could be that the EL-4 is a transformed cell line which is not comparable to a usual cell.

3.2. IL-2 and TNFα

The test was performed as described in "T cell growth induced by IL-2" and "Fibroblast toxicity induced by TNFα".

In contrast to the effect on the function of IL-1α and IL-1β, fusidin did not modify the ability of human IL-2 to activate mouse cytotoxic T lymphocytes (CTLL-2) or the ability of human TNFα to kill mouse L-M fibroblast (Fig. 7).

EXAMPLE 4

Effect of delayed addition of fusidin to IL-1-induced thymocytes

The test was performed as described in "T cell proliferation induced by IL-1α/β".

As shown in Fig. 8, addition of fusidin 5 μg/ml up to 48 h after initiation of thymocyte activation by PHA plus IL-1 was progressively less effective in reducing the co-stimulatory effect of IL-1. A similar response pattern was obtained after addition of 50 ng/ml of cyclosporin. This indicates that fusidin affects the early processes leading to T cell activation. Early start of treatment of immunoinflammatory diseases with fusidin or derivatives thereof is therefore most likely beneficial.

EXAMPLE 5

Effect of fusidic acid derivatives on IL-1-induced thymocyte activation

The test was performed as described in "T cell proliferation induced by IL-1α/β".

As shown in Table 4, only 3 fusidic acid derivatives were effective in inhibiting the thymocyte co-stimulatory effect of IL-1α and IL-1β. The ineffective derivatives are characterized by modifications in the substituents at positions 16 and 21 of the molecule suggesting that these regions of fusidic acid are necessary for the IL-1-inhibitory effect of the drug.

TABLE 4

Effect of fusidic acid derivatives on the thymocyte co-stimulatory function of IL-1

	% inhibition of			
Fusidic acid derivative	rIL-1α	P	rIL-1β	P
Fusidin	70 +/- 10	<0.05		
VD 1177	21 +/-19	ns	5 +/-9	ns
VD 1178	16 +/-13	ns	9 +/-8	ns
VD 1303	2 +/-11	ns	7 +/-3	ns
VD 1360[1]	24 +/-6	<0.05	22 +/-9	<0.05
VD 1362[1]	59 +/-10	<0.05	61 +/-15	<0.05
VD 1460[2]	46 +/-13	<0.05	58 +/-13	<0.05
VD 1546	3 +/-9	ns	14 +/-7	ns
PR1089	-10 +/-10	ns	13 +/-5	ns

Results are means +/- SEM; n=6.

[1] These analogues were identical with fusidin at the 16 and 21 positions of the molecule

[2] This analogue was also identical with fusidin at the 16 and 21 positions, except for substitution of oxygen with sulfur at position 16.

EXAMPLE 6

Fusidin-induced inhibition of the human two-way mixed lymphocyte reaction (MLC)

The test was performed as described in "Human mixed lymphocyte reaction".

MLC is the *in vitro* correlate of a transplant rejection *in vivo*. Therefore, if fusidin has a role in preventing graft rejection, it should suppress the MLC reaction. As shown in Fig. 9, fusidin indeed inhibited the two-way human MLC. The dose-response was almost identical to those obtained when testing the effects on IL-1 on thymocytes and PPD-activated human T cells as well as IL-2 production. The 50% inhibitory concentration of fusidin was 1.5-5 μg/ml. Similar suppression of the MLC was obtained with 15-50 ng/ml of cyclosporin.

EXAMPLE 7

Fusidin-induced inhibition the hybridoma growth-promoting effect of IL-6

The test was performed as described in "Hybridoma growth induced by IL-6".

IL-6, another MØ-derived peptide mediator, has been reported to mediate several functions of IL-1, including thymocyte activation (2,7,8). It was therefore of interest to see whether fusidin interfered with the function of IL-6. As shown in Fig. 10, the growth of the B9 hybridoma cell line was inhibited by fusidin. The dose response was very similar to the ones obtained when testing the effect of fusidin on the IL-1-induced growth of normal (mouse) thymocytes. Fifty % reduction in IL-6-induced growth was obtained at around 10 μg/ml of fusidin. Again, there was no evidence of fusidin-induced cytotoxicity, as demonstrated in the next experiments.

As shown in Fig. 11, the ability of fusidin to prevent IL-6-induced proliferation of B9 hybridoma cells was not caused by a generalized toxic effect on the cells. Thus, B9 cells preincubated with 30 µg/ml of fusidin, with or without rIL-6, responded normally to a subsequent challenge by IL-6 after washing the cells with medium and reconstitution without fusidin.

EXAMPLE 8

Failure to modify IL-1-induced activation of bone resorption

The test was performed as described in "Bone resorption induced by IL-1α".

As shown in Table 5, fusidin did not affect the increased release of radiolabelled Ca induced by rIL-1α. This further demonstrates the non-toxicity of fusidin in biological systems.

TABLE 5

Lack of effect of fusidin on rIL-1α-induced bone resorption

		^{45}Ca release (% of control)				
Fusidin	rIL-1α	mean	+/-	SD	n	P
-	-	1.00				(control)
10 µg/ml	-	0.95	+/-	0.08	10	ns
10 µg/ml	20 U/ml	1.49	+/-	0.12[1]	9	<0.05
-	20 U/ml	1.35	+/-	0.10[2]	8	<0.05

[1] and [2]: no significant difference between these values.

EXAMPLE 9

Fusidin-induced protection of pancreatic β-cells against the inhibition/damage afforded by IL-1

The tests were performed as described in "Insulin production by isolated rat islets".

9.1. Fusidin

Since IL-1β is considered pathogenetically important for the development of IDDM, and since IL-1β significantly reduces glucose-induced insulin production by pancreatic β-cells *in vitro*, it was of interest to test whether fusidin could restore normal β-cell function *in vitro*. As shown in Fig. 12, co-incubation of pancreatic islets isolated from normal rats with increasing concentrations of fusidin progressively normalized their glucose-induced insulin production, even in the presence of relatively high concentrations of rIL-1β. At the highest concentration of fusidin, there appeared to be a slight reduction in the protective effect of the drug. The reason is not clear but is most likely caused by the drugs ability to inhibit insulin mRNA translation (23).

9.2. Fusidic acid derivatives

A similar protective effect was not observed when testing a number of fusidic acid derivatives (Fig. 13) whereas the diethanolamine salt (3 experiments) gave similar results as fusidin. This indicates that modifications of the basic fusidic acid structure except for derivatives at the 21 position such as the salt formation are not desirable in connection with this effect.

DISCUSSION RELATED TO THE *IN VITRO* EXPERIMENTS

Immunosuppressive effects of fusidin

The data presented from the *in vitro* experiments demonstrate a dose-dependent inhibitory effect of fusidin on processes involved in lymphocyte activation. Induction of T and B lymphocytes requires several steps in addition to antigen or allogen recognition by HLA class II positive cells such as MØ or dendritic cells: 1) production and release of IL-1 and, possibly, IL-6; 2) activation of T cells by these cytokines through specific membrane receptors for IL-1 and IL-6; 3) production and release of IL-2 by T lymphocytes; 4) acquisition of receptors for IL-2 on T lymphocytes, and 5) internalization of IL-2-receptor complexes. In addition, the release from T cells of IFNγ is considered important, because this cytokine is a potent activator of MØ functions, including HLA class II expression (1,8).

Like cyclosporin (19), fusidin appears to interfere with some of these early processes. First, relatively high concentrations of fusidin inhibited production/secretion of IL-1 from LPS-PHA-activated human blood MNC. Secondly, and probably more importantly, low therapeutic concentrations of fusidin suppressed lymphocyte activation by IL-1 and IL-6, another MØ-derived activator of T- and B lymphocytes. Probably as a result of this, fusidin also inhibited the production by T cells of their own growth factor, IL-2, leading to impaired T cell proliferation after antigenic, mitogenic as well as allogeneic activation. Since IFNγ is an important activator of MØ functions, including their ability to secerete cytokines and express HLA class II molecules, the drugs ability to inhibit the elaboration of IFNγ might contribute to T cell suppression.

In all cases, the inhibitory effects were dose-dependent, and the effective concentrations of fusidin appeared to be non-toxic to the target cells. Furthermore, fusidin did not function by a general antiproliferative effect, because the IL-2-induced proliferation of CTLL-2 cells were unaffected by the drug.

The concentrations of fusidin which were shown to be effective *in vitro* seem to be clinically relevant, because therapeutic serum concentrations in the range 15-100 μg/ml may be obtained in man without significant side-effects (33). However, fusidin is bound extensively to protein *in vivo*, and this may contribute to a diminished clinical efficacy if related to the effective concentrations of the drug *in vitro*. This, however, does not appear to be of major importance in the case of fusidin, because increased concentrations of serum in the thymocyte assay only marginally affected the fusidin-induced immunosuppressive function. Moreover, several important clinical effects of fusidin are expected to take place in tissues where serum proteins are absent or present in only small quantities.

When testing fusidin in the mouse thymocyte co-stimulatory assay, the IL-1 activity was almost completely eliminated by 15 μg/ml of the drug, and a 50% inhibition was achieved at a concentration of 1.5-5 μg/ml. At all concentrations, fusidin failed to decrease the viability of the thymocytes compared with thymocytes cultured in parallel without the drug.

The mechanism by which fusidin inhibits the immunostimulatory functions of IL-1α/β is unknown. However, the drug is known to inhibit protein synthesis at the translational level (23), and an impaired protein synthesis may therefore, at least in part, explain the results. However, a complete inhibition of cytokine mRNA translation cannot by itself explain our findings. Thus, the production of TNFα by human blood MNC activated by LPS+PHA was unaffected even by 50 μg/ml of fusidin. Also, the ability of fusidin to inhibit production of the cytokines, IL-2 and IFNγ, is most likely a consequence of the IL-1α/β and the IL-6inhibitory effects of the drug rather than a direct effect on the translation of these lymphokines. Hence, IL-1 in particular is considered an essential 'second signal' for T lymphocyte activation, including production of lymphokines (1-4) the first signal being antigen or mitogen-induced perturbation of the T cell receptor/CD3 complex (1,2,11,12).

The ability of fusidin to inhibit a mixed lymphocyte reaction *in vitro* is probably also secondary to its IL-1-inhibitory effect. At

any rate, the inhibitory effect of fusidin on this *in vitro* correlate of a human allograft rejection may be important, because it suggests that the drug may be used clinically to prevent the hosts elimination of allotransplants, such as kidneys, livers, hearts, lungs, skin, bone marrow, corneae, etc. Treatment of the host with fusidin at the time the transplant is performed, and thus interference with the 'second signal' of lymphocyte activation, may also lead to specific tolerance to the grafted tissue (see above).

The ability of fusidin to interfere with the growth-promoting effects of IL-1α/β and IL-6 is also of clinical importance. Thus, IL-1 and particularly IL-6 have been implicated in the pathogenesis of several lymphoproliferative diseases, such as multiple myeloma and other plasma cell, and B- and T cell cancers (see above).

Antiinflammatory effects of fusidin

IL-1 and IL-6 are known to cause an array of biological activities in many cell types (1-4). It is therefore interesting that fusidin interfered with some but not all the various functions of IL-1 that were tested.

For example, the drug was unable to affect IL-1-induced bone resorption. On the other hand, the drug clearly protected pancreatic islet β-cells against the inhibitory/cytotoxic action of IL-1β.

The apparent inhibitory effect of fusidin on non-immunological functions of IL-1 and, possibly, IL-6 is of clinical interest. Thus, apart from being involved in lymphocyte activation and thus presumably in physiological and pathophysiological immune reactions, IL-1 and IL-6 are involved in the development and manifestations of many infectious and immunoinflammatory diseases, including AIDS, autoimmune endocrine diseases, some rheumatic diseases, etc. (Table 1, ref. 2,3,8). This, along with the ability of IL-1α/β and IL-6 to cause fever, and of IL-1α/β to evoke anorexia and cachexia, emphasizes a therapeutic role of fusidic acid and derivatives thereof apart from their usage as antibiotics.

CONCLUSIONS FOR THE *IN VITRO* EXPERIMENTS

T- and B-lymphocytes, MØ and NK cells play important roles in man's defence against microorganisms and neoplastic diseases. On the other hand, there is strong evidence for a role of these cells in the pathogenesis of certain rheumatic diseases and immunoinflammatory diseases involving the endocrine system, the skin, the gut, and many other organs. The peptide hormones (cytokines) produced by these cells, particularly IL-1α, IL-1β, IL-6 and TNFα, are potent immune stimulators as well as modulators of hepatocytes, bone cells, endothelial cells, fibroblasts and synovial tissue cells, pancreatic islet β-cells, thyrocytes, and many other cells. Treatment to prevent the production and/or function of IL-1α/β, IL-6 and TNFα may prevent the development, or ameliorate the symptoms, of many immunoinflammatory and infectious diseases. Such intervention may also prevent transplant rejection either if administered as a continuous, immunosuppressive treatment or as a timed, short-term treatment to induce specific tolerance to the grafted tissue. Fusidin, in therapeutic relevant concentrations, inhibit several immunological, growth-promoting and pro-inflammatory functions of IL-1α/β and IL-6 *in vitro*, and the patterns of these responses are strikingly similar to those caused by cyclosporin. The present discovery is therefore of importance in the systemic and local treatment of several immunoinflammatory diseases in humans, where these cytokines are considered to be of pathogenetic relevance. These diseases include the so-called autoimmune diseases (see Table 1), diseases associated with transplantation, including graft rejection and graft-versus-host disease, many infectious diseases, and many diseases characterized by pathological cell growth, including some neoplastic diseases.

In the following, a number of *in vivo* experiments are described, either based upon evidence obtained or as guidelines for the experimental work to be performed in connection with specific uses of fusidic acid or derivatives thereof. With respect to pharmacology, the pharmacology of fusidic acid and derivatives thereof for oral, parenteral and topical application appears from the scientific and patent literature mentioned in the section of "Background of the Invention". This documentation is supplemented by the following

example illustrating penetration of the drug into compartments of the eye (Example 10).

IN VIVO EXPERIMENTS

PHARMACOLOGICAL STUDY

EXAMPLE 10

Analyses From Corpus Vitreum/Subretinal Fluid

The fusidin concentration in corpus vitreum/subretinal fluid is measured after 3 days of preceeding systemic therapy prior to operation. Systemic therapy: Fusidin tablets (Leo, Copenhagen, Denmark) 500 mg 3 times every 24 hours. The therapy ends at least 6 hours before operation.

Material

1. Corpus vitreum analyses include 10 eyes of patients who are subjected to a vitreous body operation in connection with bleeding in the vitreous body provoked by diabetes mellitus.

2. Amotio operation (detachment of the retina), removal of subretinal fluid of 10 eyes in connection with retina operations.

PRE-CLINICAL STUDIES

The following examples 11-12 are based upon ongoing studies and give guidelines for preclinical studies in animal models.

EXAMPLE 11

In vivo examination of the prophylactic effect of fusidin in two animal models of diabetes mellitus

The following example is designed to examine the prophylactic effect of fusidin in BB-rats and NOD-mice (two spontaneously diabetic animal models for Type 1 (insulin-dependent) diabetes mellitus).

The study includes 40-50 animals treated with fusidin and a corresponding number of control animals treated with placebo. Breeding couples are procured and the experimental animals are supported and breed. The animals are treated with the drugs immediately after weaning and until the study is terminated after 200 days. The animals are observed daily, week-ends included, and once a week the animals are weighed and urine tests are carried out to determine glucose content. Pancreas from animals developing diabetes and from animals at the end of the study are subjected to microscopic examination to discover 1) possible infiltration of mononuclear cells in the islets of Langerhans (insulitis), and 2) to determine the number of cells producing insulin. Furthermore, the contents of insulin and cytokines in consecutively drawn serum samples are determined in order to evaluate a possible association between the development of the disease and the cytokine levels in serum.

EXAMPLE 12

The effect of fusidin on the acute-phase response induced by IL-1 and IL-6 in mice

Background

The acute-phase reaction is usually seen during acute and chronic infectious and inflammatory diseases, and in cancer, and administration of IL-1, IL-6 or TNFα reproduces this reaction (8). IL-6 and, to a lesser extent, IL-1 and TNFα induce hepatocytes to synthesize acute

phase proteins, including serum amyloid A, C-reactive protein, fibrinogen, haptoglobin, complement components and clotting factors. At the same time, the blood level of albumin decreases, as does the plasma concentrations of Fe^{2+} and Zn^{2+}, whereas the level of Cu^{2+} increases. Associated phenomena are fever, leukocytosis and induction of sleep. The elevated level of fibrinogen, especially if accompanied by anemia, causes an increased sedimentation rate of the blood, a commonly used clinical parameter of 'inflammation'.

The above mentioned clinical picture is often associated with disturbances in carbohydrate-, lipid- and protein metabolism resulting in wasting (cachexia). In rare situations, the acute-phase reaction may progress and lead to clotting abnormalities, shock, and death. It was previously thought that microbial products such as endotoxins were directly responsible for these symptoms, if triggered by bacterial infection. This is now known to be incorrect, because endotoxins are potent inducers of MØ IL-1, IL-6 and TNFα, and all pathophysiological processes associated with endotoxin-induced shock can be reproduced by injection of TNFα and, to a lesser extent, by IL-1 (2).

Design

Pilot study using a mouse model (25).

12.1. Interleukin 1α-induced response

Fusidin, 15 mg, and solvent alone (control) are administered i.v. into 5 plus 5 (controls) female BALB/c mice (8-10 weeks old). After 30 min, 10 μg of human rIL-1α in 25 μl pyrogen-free isotonic saline is given i.v. After 24 h, blood is drawn for measurements of the mouse acute phase reactant haptoglobin.

Depending upon these results, the dosage of fusidin and rIL-1α will be altered in order to substantiate the dose-response curve of the effect of fusidin on the IL-1-induced acute-phase response in mice.

12.2. Interleukin 6-induced response

The experimental approach outlined above will be used, except that human rIL-6, 10 μg, will be used.

Again, the dose-response curve of the effect of fusidin will be established.

12.3. Effect of fusidin analogs on IL-1α- or IL6-induced acute-phase reactions in mice

Similar experimental approach as above, except that fusidin analogs will be used instead of fusidin.

The following examples 13-18 concern clinical experience gained by certain treatments of patients with fusidin and give guidelines for controlled clinical studies based upon the evidence gained from these pilot studies.

CLINICAL STUDIES

EXAMPLE 13

The following studies are designed to demonstrate the effect of fusidic acid eyedrop treatment as a prophylactic treatment against inflammation after eye surgery.

13.1. Clinical Examination of the Antiinflammatory Effect of Fusidin in the Postoperative Period after Uncomplicated Operation for Cataract

Material: Patients aged over 60 operated for cataract with extraction of the lens and subsequent implantation of an artificial lens in the posterior chamber of the eye corresponding to 20 eyes.

Exclusion: Patients with eyes with trauma, glaucoma, retinovascular bleeding or thrombosis, corioretinal inflammatory and non-inflamma-

tory sight-threatening diseases, diabetes mellitus (type I), collagenosis.

The group treated with fusidin includes patients corresponding to 10 eyes.

The control group includes patients corresponding to 10 eyes treated with ultralanum with chloroamphenicol.

Method: Randomized therapy between the two preparations of 20 eyes in a 3 week period postoperatively starting from the first postoperative day.

Dosage: Ultralanum with chloroamphenicol eye drops 4 times daily. Fucithalmic eye drops 4 times daily.

Evaluation parameters:

1. Visual acuity: Examined at weeks 1, 2 and 3 after the cataract operation.

2. Biomicroscopy is carried out at days 2, 5, 14 and 21 after operation. During biomicroscopy, a more detailed data recording would be carried out.

Eye pressure is measured at weeks 1, 2 and 3 after operation.

The examination of corpus vitreum and the corioretinal status is carried out at weeks 1, 2 and 3 after operation, and subjective data in the postoperative period are recorded including the patients' evaluation of the treatment with fucithalmic/ultralanum with chlorampfenicol, e.g. problems with dripping of eyes, eye pain, duration of eye pain, duration of sight reduction, if any, after dripping of eyes, etc.

Exclusion during treatment: Increased intrabulbar inflammation, (sight-threatening), obvious panophthalmia, non-compliance.

13.2. Clinical examination of the antiinflammatory effect of fusidin in the postoperative period after uncomplicated laser surgery

Material

Patients admitted for laser surgery due to e.g. loosening of the retina, retinal biopsy or diabetic retinopathy.

The group treated with fusidin includes patients corresponding to 10 eyes.

The control group includes patients corresponding to 10 eyes treated with conventional therapy (glucocorticoid).

Method: Randomized therapy between the two preparations in a 3 week period postoperatively starting from the first postoperative day.

Dosage: Fusidin tablets 500 mg x 3 in 2 weeks starting at the day of the operation.

Evaluation parameters:

1. Visual acuity: Examined at weeks 1, 2 and 3 after the laser surgery operation.

2. Biomicroscopy is carried out at days 2, 5, 14 and 21 after operation. During biomicroscopy, a more detailed data recording will be carried out.

The examination of corpus vitreum and the corioretinal status is carried out at weeks 1, 2 and 3 after operation, and subjective data in the postoperative period are recorded including the patients' evaluation of the treatment with fusidin.

EXAMPLE 14

Treatment with Fusidin of Non-infectious, Immunoinflammatory Uveitis

Background

Severe uveal tract inflammation (uveitis) is responsible for a large percentage of the visually handicapped patients in developing and developed countries. Most of the cases of uveitis in developed countries are classified as idiopathic and are presumed to have an underlying autoimmune cause. The treatment in these cases is mainly based on the use of glucocorticoids, cytotoxic drugs or cyclosporin. In many of the cases, however, the treatment only delays the loss of vision side-effects (Cushing's syndrome, and severe bone-marrow depression and nephropathy (cyclosporin) may force withdrawal of therapy with resulting loss of vision.

14.1. Pilot studies

In the following Tables 6 and 7 are shown the results from two pilot studies. In the first study, three patients are included corresponding to treatment of five eyes. The treatment was converted from cyclosporin to fusidin (0.5 g 3 times daily given as tablets). In the second study, four patients are included (8 eyes). The patients were treated with fusidin (0.5 g 3 times daily given as tablets) without earlier cyclosporin medication.

TABLE 6

Pilot study: Severe, sight-threatening uveitis

Three patients (5 eyes) - conversion from cyclosporin (CyA) to fusidin (Fus)

Id	Case history	CyA therm.	Prednisolone (mg/day) (before	- last)
NJN	One eye blind	5 years	10	7.5
JK	Relapse on CyA + pred. 7.5 mg/day 2 x relapse on pred.pulse Mb. Cushing	1.5 years	0	0
MN		2.5 years (nephropathy)	100	15

TABLE 6 (continued):

Id	oc	Visual acuity (before	- last)	Acute infl. fundal changes (before	- last)	Notes
NJN	sin	6/9	6/9	+	-	
JK	dxt.	6/9	6/18	-	-	2 x vitrectomy during Fus. therapy without relapse
	sin.	6/36	6/36	+	-	
MN	dxt.	6/6	6/6	+	-	
	sin.	<3/60	<3/60	++	-	

Comments: All 3 patients (5 eyes) benefited from fusidin therapy. Deterioration in visual acuity in 1 eye was caused by vitreal haze secondary to a vitrectomy. Two vitrectomies were performed during Fus. therapy *without* flare-up of disease activity! None of the patients had side-effects of Fus. All. 3 patients had severe side-effects caused by the long-term treatment with moderate/high doses of glucocorticoids and CyA.

TABLE 7

Pilot study: Severe, sight-threatening uveitis

Four patients (8 eyes) - fusidin (Fus) treatment (no cyclosporin)

Id	Case history	Fus therm.	Prednisolone (mg/day) (before - last)
BNJ		7 months	50 7.5/50 20 (relapse)
VCK	Relapse on pred. 30 mg/day Mb. Cushing	7 months	30 10
FVT		7 months	60 10/60 0/30 20 (2 relapses)
GHP	Relapse on pred. 30 mg/day	3 months	60 10/40 25 (relapse)

TABLE 7 (continued):

Id	oc	Visual acuity (before	- last)	Acute infl. fundal changes (before	- last)	Notes
BNJ	dxt.	6/60	3/60	++	-	Plus Fus. eye-drops
	sin.	6/9	>6/9	+	-	Relapse at pred. 7.5 mg/day
VCK	dxt.	6/9	6/9	+	-	Subj. improvement after 2 days of Fus.
	sin.	6/9	6/9	+	-	
FVT	dxt.	6/6	6/6	+	-	2 relapses at pred 10 and 0 mg/day, resp.
	sin.	6/18	6/18	+	-	
GHP	dxt.	6/9	6/9	+	-	Relapse at pred 10 mg/day
	sin.	6/18	6/18	+	-	

Comments: Even though the evalation of the clinical response is complicated by concomitant therapy with glucocorticoids, all 4 patients (8 eyes) benefited from fusidin therapy. Thus, there were no signs of acute chorioretinal inflammation, and 2 patients were able to lower the dosage of glucocorticoid. However, attempts to completely withdraw glucocorticoid treatment resulted in relapse in 3 patients. Similar relapses are seen if glucocorticoid therapy is withdrawn from CyA-treated patients with severe uveitis. None of the patients had side-effects attributed to fusidin.

14.2. Clinical Examination of the Antiinflammatory Efficacy of Fusidin in Patients with Uveitis Anterior

Material: Patients with acute onset or recurrence of uveitis anterior corresponding to 20 eyes.

Group 1 includes patients corresponding to 10 eyes treated with fucithalmic eyedrops 6 times daily.

Group 2 is treated with glucocorticoid eyedrops 6 times daily (e.g. Maxidex®, from Alcon, Texas, U.S.A.)

Both groups are treated with atropine eyedrops (e.g. from DAK, Copenhagen, Denmark) 1% twice daily.

Duration of treatment: 3 weeks.

Clinical control: At least once a week.

Exclusion: Uveitis as a result of bacterial/viral infections, ongoing systemic therapy for collagenosis, aggravation of uveitis during actual treatment, and non-compliance.

14.3. Multicenter study of the efficacy of fusidin in sight-threatening, non-infectious, immunoinflammatory, posterior uveitis

Aims of Study

To compare efficacy, safety and tolerability of oral fusidin with conventional therapy (corticosteroids) for the treatment of sight-threatening uveitis.

Study parameters are:

- Number of patients withdrawn from study medication because of contraindications to continued therapy
- Visual acuity
- Parameters of inflammatory activity
- Fluorescein angiography
- Number of relapses
- Side-effects, safety parameters
- Global evaluation of efficacy and tolerability by investigator and patient.
- Immunological studies, including measurements of blood levels of cytokines.

Type of Study

Multicenter, open, randomized, controlled, parallel-group study with "masked" opthalmologist for unbiased recording of symptoms in uveitis (comparison of fusidin vs. conventional therapy).

Patients

Number: Initially 20 patients. At this stage, decision will be made whether to continue.

Starting criteria

Active uni- or bilateral ocular involvement.

Exclusion criteria

Terminal stage of disease with nonreversible degeneration of retina in which no active lesion is observed.

Patients who are suffering from myosis or cataract which diseases made it impossible to note the posterior pole in both eyes will be excluded.

Ongoing infectious disease, hepatic dysfunction, i.e. elevation 2.5 above upper limit of normal value of either ALAT, ASAT, bilirubin (direct and indirect) and total protein, non-compliance.

General Study Outline

Patients on corticosteroids and/or cytostatic agents should have these drugs withdrawn before definite selection and before randomization.

In consecutive order, patients should be randomized to either fusidin (monotherapy) or conventional therapy.

Two investigators should take part in the study. Investigator 1 should know the assigned treatment, whereas the investigator 2 (ophthalmologist) should be masked in this respect. Investigator 1 should evaluate safety parameters, side-effects and should prescribe medication. Investigator 2 should evaluate only the efficacy parameters. He should not have access to other data.

The assigned therapy should be maintained unless contraindications occur. Patients randomized to conventional therapy should start on monotherapy with corticosteroids. If after a minimum of 4 weeks, the response is inadequate, fusidin therapy should be initiated (in combination with low-dose corticosteroids). If, after further 4 weeks, the response is inadequate, fusidin should be replaced with cyclosporin therapy, and the patient should be withdrawn from the study.

Patients should be investigated at baseline, at weeks 1, 2, months 1, 2, 3, 4, 6, 9, 12 and thereafter at 6 monthly intervals if the disease is quiescent or at any time if acute attacks occur.

First full analysis of data should be performed on 6-months data of the first 20 patients, and thereafter at 12 months intervals.

The only concomitant medication for uveitis allowed are glucocorticoids and cycloplegic eye drops. Subconjunctival glucocorticoid injections are not allowed.

A) Test Medication

Fusidin tablets 0.5 g 3 times daily

B) Conventional Therapy-Group

1.5 mg prednisolone (e.g. from DAK, Copenhagen, Denmark) per kg per day for a week. The dose will be decreased gradually to reach a maintenance level of 5 to 10 mg prednisolone per day. In case of relapse, the dosage will be increased.

Contraindications for Continued Therapy (for both treatment groups):

- Visual acuity: If after maximum therapy of at least two weeks, the visual acuity falls two lines below baseline at the initiation of therapy) on two successive days in either affected eye.

- Inflammation: If, after maximum therapy of at least two weeks, the inflammatory activity as determined by a second observer, is worse and the visual acuity is not improved in either eye.

- If the disease process progresses into the macula which in the opinion of a second observer might lead to permanent loss of vision in either eye.

- Toxicity or side-effects

  a) Impaired hepatic function, such as any value 2.5 times that of the upper limit of normal
  b) Allergic reactions to fusidin
  c) Side-effects felt by the patient to be of such a nature that it is impossible for him/her to continue.

Disease activity

Patients will be evaluated at baseline and at weeks 1, 2 and months 1, 2, 3, 4, 6, 9, 12, and thereafter at 6-monthly intervals during at least 1 year if disease is quiscent or at any time if acute attack occur.

Concomitant medications: Any concomitant medications will be listed during the pretreatment evalution and at each subsequent evaluation.

Ophthalmological evaluations by "masked" ophthalmologist:

a) Visual acuity

b) Inflammation, anterior chamber flare, vitreal haze, and opacity

c) Other ocular findings

d) Flurorescein angiography (only to be done when media allows and at months 3, 6 and 12)

Laboratory tests

a) Blood: Hemoglobin, hematocrit, WBC count and differential, platelets, sedimentation rate, total protein, total bilirubin, alkaline phosphatase, ALAT, ASAT, potassium and creatinine.

b) Qualitative urinalysis (protein, sugar, sediment).

c) At entry to study only: Pregnancy test.

Immunological Investigations (optional):

Serum parameters:

a) Serum protein electrophoresis

b) Ig levels

c) Serum IFNγ, IL-2, IL-2 receptor, IL1α/β, TNFα

Cellular parameters:

a) Blood lymphocyte and monocyte analyses - total count and subsets

b) Histocompatibility antigens - HLA-DR (only at time 0).

Autoimmune responsiveness:

a) Antinuclear antibodies

b) Rheumatoid factor

Functional immune capacities:

a) Background levels and responses to LPS and PHA measured by proliferation assays and cytokine production

b) Auto-antibodies for cytokines (IL1α and TNFα)

Withdrawals / Drop-outs

Withdrawals are patients who for reasons related to test medication (contraindications as specified) stop further therapy. They should be fully analysed and should not be replaced by new patients. At time of withdrawal, a complete evaluation should be performed.

Drop-outs are patients lost to follow-up or non-complying to protocol. They should be replaced (next free patient number).

EXAMPLE 15

Fusidin treatment of Crohn's disease

Background

It has been established that cyclosporin, is effective against Crohn's disease (46). However, the treatment can only be given in small doses and in short periods of time due to the risk of dangerous side-effects.

Aims of study: Examination of the gastrointestinal absorption of fusidin and the possible effect of fusidin in the treatment of Crohn's disease.

Type of study: Open pilot study

Patients

The initial material includes a total of 6 patients with Crohn's disease.

Starting criteria

- Active Crohn's disease where medical treatment is planned.

- Any prednisolone treatment should not exceed 20 mg per day and must be kept unchanged for at least 2 weeks.
- Any salazopyrin (SASP) or 5-aminosalicylic acid (5-ASA) treatment should also be kept unchanged for at least 2 weeks.

Exclusion criteria

- Patients receiving any other treatment of Crohn's disease.
- Patients having received cytostatic treatment within the last 2 weeks.
- Patients with a known hypersensitivity to fusidin.
- Patients with known, serious liver diseases.
- Patients planning to become pregnant or being pregnant.

General Study Outline

Fucidin tablets 0.5 g 3 times daily for 4 weeks. If no positive effect is registered, the patient should be excluded from the test.

If a positive effect is registered, treatment is carried on for another 4 week period. In case the disease is aggrevated and other treatment is needed, the patient can at any time be excluded from the test.

Other treatment

If the patient is receiving SASP (salicylazosulfapyridine) / 5-ASA (5-aminosalicylic acid) treatment by the start of the test, dosage should be kept unchanged. Concerning prednisolone, see below.

Disease activity

The disease activity is measured by means of the modified "Grading Score" a.m. (46). The effect of the treatment is defined as a positive total score. The reduction of the dosage of prednosolone can be included as an individual treatment aim. As secondary activity aims, P-orosomucoid concentration and "Crohn's Disease Activity Index" (CDAI) are used.

Clinical control with blood sampling should be carried out after 0, 2, 4, 8 and 12 weeks of treatment and 4 weeks after ended therapy and at any other time depending upon clinical status.

Laboratory tests

Blood: Sedimentation rate, orosomucoid, hemoglobin, hematocrit, bilirubin, ALAT, ASAT, alkaline phosphatase.

Furthermore, samples are taken (5 ml serum, 5 ml EDTA-plasma and 5 ml heparin plasma) for determining the fusidin concentration - the patient is not allowed to take his morning dosage until after the blood samples have been taken - and plasma levels of cytokines and cytokine autoantibodies.

EXAMPLE 16

Fusidin in treatment of acute graft-versus-host disease (acute GvHD).

Background

Acute GvHD is a symptom complex appearing 8-40 days (median 14 days) after allogeneic bone marrow transplantation as a consequence of the reaction of donor T-lymphocytes against recipient antigens, especially transplantation antigens in the HLA-system. This occurs in 70% of the patients with HLA-identical sibling donors but is more frequently observed in HLA incompatible family donor or HLA-identical unrelated donor.

The following grading is valuble interims of the prognosis and therapeutic response:

Grade I (slight grade): Macular or confluent exanthema. Diarrhoea not more than 500 ml per 24 hours.
Bilirubin < 25 mikromol/liter.
Grade II (moderate grade): Exanthema as well as diarrhoea 500-1000 ml per 24 hours and/or bilirubin > 25 mikromol per liter
Grade III and IV (heavy grade): Exanthema and diarrhoea more than 1000 ml per 24 hours and/or bilirubin > 50 mikromol per liter.

The occurence of acute GvHD in moderate to heavy grade is combined with increased lethality after allogeneic bone marrow transplantation. Lethality is especially due to infections as a result of delayed immunological reconstitution. This is caused both by the GvHD itself and by the fact that these patients often receive further immunosuppressive treatment with prednisolone and/or anti-thymocyte globulin.

Presently applied treatment strategy

All patients are treated prophylactically against acute GvHD. This includes cyclosporin (day -1 to day +180), possibly supplemented with methotrexate i.v. (day 1, 3, 6 and possibly 11), depending upon the estimated risk of developing acute GvHD.

Supplementary treatment against GvHD is carried out in the following situations:

1. *Grade I* (slight) GvHD which is subjectively very annoying, or which is not reduced after app. one week's observation.
2. Quickly progressing *grade II* (moderate) GvHD.
3. *Grade III* (heavy) GvHD.

Glucocorticoids are used, possibly supplemented with anti-thymocyte globulin. The dosage of cyclosporin is also increased.

Aim and type of study

Pilot examination to evaluate the effect of fusidin in patients treated with allogeneic bone marrow transplantation who develop acute GvHD despite adequate prophylaxis with cyclosporin and, optionally, methotrexate. If fusidin reduces the development of GvHD, a clinically important reduction in glucocorticoid and cyclosporin consumption and, hence, a reduction in side- effects can be obtained (e.g. infections and nephropathy).

Patients: 10 patients receiving allogeneic bone marrow transplants because of leukemia, aplastic anemia, or other life-threatening marrow dysfunction

Exclusion criteria

- Patients under 18 years of age.
- Patients who according to the above-mentioned criteria should be treated with glucocorticoid.
- Patients receiving glucocorticoid and/or anti-thymocyte globulin before or at the time of transplantation.

Note:
Before fusidin treatment is carried out, adequate cyclosporin prophylaxis should be carried out:

a. If the results of the latest cyclosporin concentration test are below therapeutic level, the cyclosporin dosage should be increased and the effect should be awaited (24 hours).
b. If the results of the latest cyclosporin concentration test is at an adequeate level, but the patient is in the meantime beginning to suffer from diarrhoea and/or vomiting which can lead to non-absorption of cyclosporin, peroral cyclosporin must be substituted with intraveneous cyclosporin and the effect should be awaited (24 hours).

General study outline

In patients eligable for treatment with fusidin (see above):

- Blood samples are taken for measuring the cyclosporin concentration (valley-value).
- Fusidin tablets or mixture or, in cases of moderate/severe diarrhoea, fusidin for i.v. injection, 12 mg/kg 3 times daily in the first 2 days, thereafter 8 mg/kg 3 times daily.

If the fusidin treatment is considered efficaceous, treatment is continued for 10 days whereafter fusidin is withdrawn.

If progression occurs in spite of fusidin treatment, the drug is withdrwan and conventional therapy is instituted (see above).

Disease activity

For each individual patient, the spread of the exanthema is recorded along with diarrhoea volume and body temperature

Laboratory tests

Blood: Sedimentation rate, orossomucoid, hemoglobin, bilirubin, ALAT, ASAT, alkaline phospatase. 5 ml serum and 5 ml EDTA-plasma are taken for measurements of cytokines and autoantibodies to cytokines.

EXAMPLE 17

Fusidin in treatment of multiple myeloma

Clinical effect of fusidin in early management of multiple myeloma

Aim of study

To gain preliminary experience with fusidin in the treatment of patients with multiple myeloma

Specific aims are to etablish:

- Dosage schedule of fusidin.
- Safety and tolerability of fusidin.
- Effects of fusidin on immunoinflammatory parameters.

Depending upon the results of this pilot study, a decision should be made whether to start a controlled study.

Type of study

Open, uncontrolled pilot study.

Patients

10 adults of both sexes.

The diagnosis of multiple myeloma is established according to conventional criteria, i.e. the classic triade: Marrow plasmocytosis (>10 percent), lytic bone lesions, and a serum and/or urine M component, or plasmocytosis associated with a progressive increase in the M component over time or if extramedullary mass lesions develop.

Study medication

Fucidin tablets 0.5 g three times daily for at least 3 weeks

Investigations:

At entry, the following data are recorded:

- M component
- marrow biopsy, radiogram of the skull
- concomitant disease(s) and medication

Laboratory tests:

Blood: Haemoglobin, RBC, WBC, and differential count, platelets; serum creatinine, bilirubin, alkaline phosphatase, ALAT, ASAT, electrolytes, se-calcium, IGA, IgG, IgM, M component.

Urine: 24-h-proteinuria, glucose, M component.

Immunological tests:

Blood levels of cytokines and *in vitro* production of IL-6

Follow-up investigations:

The study runs for minimum 3 weeks and maximum 3 months. Data which will be collected at weekly intervals during the first 3 weeks, and then at 2-4 weekly intervals:

- Side-effects which can be ascribed to fusidin.
- Symptoms and signs of multiple myeloma and concomitant disease(s).
- Details of all therapy.

Laboratory parameters: Creatinine, creatinine clearance, liver function tests, hematology, urine (24 h proteinuria), M component.

The immunological tests should be repeated at least once monthly.

- True plasma levels of fusidin (blood drawn in the morning before first dosage of fusidin) should be performed at bi-weekly intervals.

Withdrawals/drop-outs

Withdrawals are defined as patients who discontinue therapy either because of side-effects or inadequate efficacy. These patients should be fully analysed and data safety and efficacy required also after withdrawal.

Drop-outs are patients lost to follow-up, not complying to instructions or otherwise violating the protocol. These patients should be replaced by new patients.

EXAMPLE 18

Clinical effect of fusidin in early management of rheumatoid arthritis, juvenile rheumatoid arthritis, polymyalgia rheumatica and systemic lupus erythematosus (SLE)

18.1. Effect of fusidin in moderately active cases of systemic lupus erythematosus (SLE) as an adjunct to glucocorticoid therapy

Background

The use of glucocorticoids has improved the outcome of systemic lupus erythematosus (SLE), particularly if accompanied by renal involvement. The addition of other immunosuppressive drugs, including cytostatic drugs, has been advocated in some cases. However, there is a need for a treatment complementary to glucocorticoids in SLE because:

a. Some patients, particularly patients with systemic vasculitis, do not respond to corticosteroids alone.

b. Many patients are in need of high dose (>10 mg prednisolone per day) corticosteroid treatment: They relapse as soon as the drug is progressively diminished or stopped.

c. Severe side-effects of high dose glucocorticoid therapy.

Aim of study

To gain preliminary experience with fusidin in the treatment of SLE patients who, despite prolonged therapy with low doses of gluco-corticoids, (≤ 10 mg prednisolone per day), show signs of clinical activity.

Specific aims are to establish:

- Dosage schedule of fusidin.
- Safety and tolerability of fusidin.
- Effects of fusidin on immunoinflammatory parameters.

Open, uncontrolled pilot study.

Patients

10 adults of both sexes.

Inclusion criteria

The SLE diagnosis should fulfil four or more of the American Rheumatism Association's criteria for the classification of SLE at the time of diagnosis. Patients who, despite receiving ≤ 10 mg of prednisolon daily for ≥ 3 months, present with subjective and/or objective signs of ongoing disease activity.

Exclusion criteria

- Patients receiving complementary treatment (e.g. cytostatic drugs) for SLE.

- Patients who are not treated with glucocorticoids.
- Pregnancy and patients in child-bearing age not practising effective birth control.

Study medication

- Fucidin tablets 0.5 g three times daily for at least 3 weeks.
- Prednisolone (e.g. from DAK, Copenhagen, Denmark). The dosage should be the mean daily dosage (≤ 10 mg per day) given in the previous three months. This dosage must be kept unaltered throughout the study.

Investigations

At entry the following data should be recorded:

- History of SLE and subjective and objective signs of SLE.
- Concomitant disease(s) and medication.

Laboratory tests:

Blood: Haemoglobin, RBC, WBC, and differential count, platelets; serum creatinine, bilirubin, alkaline phosphatase, ALAT, ASAT, electrolytes.

Urine: 24-h-proteinuria, glucose.

Immunological blood tests:

- Antinuclear and anti-DNA antibodies.
- IgG, IgA, IgM.
- Coombs'test, rheumatoid factor.
- B lymphocyte level (membrane immunoglobulin).
- T lymphocyte level and subpopulations (CD4, CD8).
- Levels of cytokines and in vitro production of IL-2, LT (lymphotoxine (TNFβ) and IFNγ.
- Complement C3 and C5a.

Follow-up investigations:

The study runs for minimum 3 weeks and maximum 3 months. Data which will be collected at weekly intervals during the first 3 weeks, and then at 2-4 weekly intervals:

- Side-effects which can be acribed to fusidin.
- Symptoms and signs of SLE and concomitant disease(s).
- Details of all therapy.

Laboratory parameters: Creatinine, creatinine clearance, liver function tests, hematology, urine (24-h- proteinuria).

The immunological tests will be repeated at least once monthly.

- Trough plamas levels of fusidin (blood drawn in the morning before first dosage of fusidin) will be performed at bi-weekly intervals.

Withdrawals/drop-outs

Withdrawals are defined as patients who discontinue therapy either because of side-effects or inadequate efficacy. These patients will be fully analysed and data safety and efficacy required also after withdrawal.

Drop-outs are patients lost to follow-up, not complying to instructions or otherwise violating the protocol. These patiens will be replaced by new patients.

18.2: Effect of fusidin as an immunoinflammatory modulator in rheumatoid arthritis given immediately after diagnosis

Background

Rheumatoid arthritis is a common disease of unknown cause. The inflammatory processes in rheumatoid arthritis include increased production of synovial fluid, activation and proliferation of cells in

the synovial membrane, destruction of articular cartilage and bone, and repair processes resulting in fibrosis, ectopic calcification and metaplastic bone formation.

Polymorphonuclear leukocytes and macrophages (MØ) play a critical role in all these processes (35, 36). Immunocompetent cells are also involved, and in severe cases the inflamed synovial tissue may resemble a lymphoid organ with germinal centers of B lymphocytes surrounded by T lymphocytes. Generally, a relatively large proportion of the infiltrating T cells bear membrane markers characteristic for activated T cells, and such cells are often found in the blood as well (35). The presence of plasma cells and immune complexes in the synovial tissue and the frequent finding of autoantibodies, particularly anti Fc-IgG rheumatoid factors, and hypergammaglobulinemia also suggests that antibody-mediated reactions are involved in the disease processes. However, these humoral reactions may reflect that the cellular infiltrate in the synovial tissue is dominated by activated T cells of the helper phenotype, and these cells may trigger B cells to produce antibodies in an uncontrolled manner. Many investigators believe that in rheumatoid arthritis, the host responds to an exogenous antigen, or an endogenous antigen rendered immunogenic in an aberrant manner, to generate an inappropriate cellular as well as humoral immune response.

IL-1 and, possibly, IL-6 and TNFα seem to play a central role in the processes leading to tissue damage in rheumatoid arthritis and related rheumatic diseases (8). Thus, injection of IL-1 and TNFα in knee joints of normal rabbits causes similar biochemical changes as those seen in *early* rheumatoid arthritis (37), and blood levels of IL-1 and IL-6 closely parallels subjective and objective disease activity in patients with rheumatoid arthritis (38).

The evidence that cyclosporin is effective in rheumatoid arthritis, both when experimentally induced (39) and in clinical cases (40-43), further support the notion that IL-1 and/or IL-6 may trigger the initial immunoinflammatory processes which eventually causes joint and bone destruction. Unfortunately, significant adverse effects of

cyclosporin have been noted, and alternative therapies are therefore advocated.

Aim of study

To gain preliminary experience with fusidin in the treatment of patients with newly diagnosed rheumatoid arthritis.

Specific aims are to establish the dosage schedule, safety and tolerability of fusidin and effects of the drug on immunoinflammatory parameters in rheumatoid arthritis.

Open, uncontrolled pilot study.

Patients

10 adults of both sexes

Inclusion criteria

The diagnosis should fulfil four or more of the American Rheumatism Association's criteria for the classification of rheumatoid arthritis at the time of diagnosis. Patients must not have received medication other than non-steroidal antiinflammatory drugs (NSAID) (in particular glucocorticoids, gold salts, penicillamine, cytostatic drugs), and the duration of active disease should not exceed 12 months.

Exclusion criteria

- Patients receiving treatment other than NSAID.
- Pregnancy and patients in child-bearing age not practising effective birth control.

Study medication

- Fucidin, tablets 0.5 g three times daily for at least 2 months.

Investigations

At entry the following data will be recorded:

- History of disease and subjective and objective signs of rheumatoid arthritis.
- Concomitant disease(s) and medication.

Laboratory tests

Blood: Haemoglobin, RBC, WBC, and differential count, platelets; serum creatinine, bilirubin, alkaline phosphatase, ALAT, ASAT, electrolytes.

Urine: protein, glucose.

Immunological blood tests:

- Antinuclear and anti-DNA antibodies.
- IgG, IgA, IgM.
- Rheumatoid factor.
- B lymphocyte level (membrane immunoglobulin).
- T lymphocyte level and subpopulations (CD4, CD8)
- Levels of cytokines and in vitro production of IL-2, LT and IFNγ.
- Complement C3 and C5a.

Follow-up investigations:

The study runs for minimum 2 months and maximum 6 months. Data which will be collected at bi-weekly intervals during the first 2 months, and then at 4 weekly intervals:

- Side-effects which can be ascribed to fusidin.
- Symptoms and signs of rheumatoid arthritis and concomitant disease(s).
- Details of therapy.

Laboratory parameters: Creatinine, liver function tests, hematology, urine (protein, glucose).

The immunological tests should be repeated at least once every second month.

- Trough plasma levels of fusidin (blood drawn in the morning *before* first dosage of fusidin) should be performed at bi-weakly intervals during the first two months and then at four weeks intervals.

Withdrawals/drop-outs

Withdrawals are defined as patients who discontinue therapy either because of side-effects or inadequate efficacy. These patients should be fully analysed and data on safety and efficacy are required also after withdrawal.

Drop-outs are patients lost to follow-up, not complying to instructions or otherwise violating the protocol. These patients should be replaced by new patients.

18.3. Other connective tissue diseases:

Since the term rheumatoid arthritis applies to a syndrome without known cause, it is difficult to ascertain whether the pathogenesis of this connective tissue disease differs markedly from related and often overlapping clinical syndromes. Thus, other connective tissue diseases, such as juvenile rheumatoid arthritis, psoriatic arthritis, reactive arthritis (Reiter's syndrome), polymyalgia rheumatica, systemic sclerosis, and sarcoidosis may be pathogenetically related. That similar disease mechanisms may be operative in several or all of these diseases is strengthened by the fact that patients with different connective tissue diseases benefit from therapy with the T cell-selective immunosuppressive drug cyclosporin (44). The treatment of these diseases, however, is often unsatisfactory, and almost always accompanied by side-effects.

The clinical studies in connection with these diseases should be performed analogously to the protocols outlined above.

LEGENDS TO FIGURES

Fig. 1: Cellular interactions and cytokine production during antigen presentation to T- and B lymphocytes. The cytokines produced by both immune and non-immune cells may enter the blood stream and act as hormones which affect the functions of distant tissues. Modified from (3).

MØ: macrophage. Th: T helper lymphocyte. B: B lymphocyte. F: fibroblast. NK: natural killer cell. DR: MHC class II molecule. IL-1 IF: interleukin 1-inducing factor.

Fig. 2: Effect of fusidin on IL-1 and TNFα production. The percentage of inhibition is shown as a function of the final test concentration of fusidin. * : $P<0.01$, n=12 (IL-1) and n=6 (TNFα).

Fig. 3: Effect of fusidin on IL-2 and IFNγ production. The percentage of inhibition is shown as a function of the final test concentration of fusidin. * : $P<0.05$, n=6.

Fig. 4: Effect of fusidin on IL-1 (mouse thymocytes). The percentage of inhibition is shown as a function of the final test concentration of fusidin. * : $P<0.01$, n=8.

Fig. 5: Effect of fusidin on IL-1 (human lymphocytes). The percentage of inhibition is shown as a function of the final test concentration of fusidin. * : $P<0.05$, n=5. PPD: purified protein derivative of tuberculin (antigen). PHA: phytohemagglutinin (nonspecific, polyclonal T lymphocyte activator).

Fig. 6: Effect of fusidin on IL-1 (mouse thymocyte cell line, EL 4). The percentage of inhibition is shown as a function of the final test concentration of fusidin. * : $P<0.05$, n=7.

Fig. 7: Effect of fusidin to inhibit IL-2 and TNFα activities. The percentage of inhibition is shown as a function of the final test concentration of fusidin. n=4 (IL-2) and n=6 (IFNγ), respectively.

Fig. 8: Effect of delayed addition of 5 μg/ml of fusidin to PHA-activated murine thymocytes.

Fig. 9: Effect of fusidin on the two-way mixed lymphocyte reaction. The percentage of inhibition is shown as a function of the final test concentration of fusidin. * : $P<0.01$, $n=10$.

Fig. 10: Effect of fusidin on IL-6 (B9 cells). The percentage of inhibition is shown as a function of the final test concentration of fusidin. Human rIL-6 was added to the test cells at a concentration of 1 U/ml. * : $P<0.02$, $n=7$.

Fig. 11: Reversibility of fusidin-induced inhibition of IL-6 activity (B9 assay). The test cells were treated as indicated. The cells were treated with 18 U/ml of rIL-6 with/without 30 μg/ml of fusidin, as indicated (PRE). After 2 days, the cells were washed three times and cultured further for 2 days with/without 30 μg/ml of fusidin, as indicated (POST). The assay for IL-6 activity was performed during the last 2 days of culture in the presence of the indicated levels of rIL-6.

Fig. 12: IL-1β induced inhibition of insulin secretion (% of control: Insulin secretion in cultures kept in medium alone without IL-1). Protection afforded by fusidin. The rat pancreatic islets of Langerhans were precultured for 6 days. The islets were then washed and added fresh medium, containing 11 mmol/l of glucose, and rIL-1β +/- fusidin at the indicated concentrations.

Fig. 13: Inability of fusidin analogs to protect against IL-1β induced β-cell damage. The culture conditions were the same as in Fig. 12, except that only one concentration of the fusidin analogs were tested.

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CLAIMS

1. The use of fusidic acid or a derivative thereof for the manufacture of a composition for substantially inhibiting a biological effect in a human related to a cytokine for prophylaxis or treatment of a condition related to disturbances of a cytokine system, such as

for the manufacture of a composition for prophylaxis or treatment of diabetes mellitus, in particular insulin-dependent diabetes mellitus (type 1), in particular for the prevention of progression of diabetes mellitus (type 1), especially substantially immediately after the first diagnostic establishment of diabetes mellitus, or for prophylaxis after establishment of being in a high risk group of developing diabetes mellitus (type 1), or

for the manufacture of a composition for the treatment of endogenous uveitis, or

for the manufacture of a composition for the prevention of the inflammation after eye surgery such as cataract operation or laser surgery, or

for the manufacture of a composition for treatment of progression of arthritis such as rheumatoid arthritis, psoriatic arthritis or Reiter arthritis substantially immediately after the first diagnostic establishment of arthritis, or

for the manufacture of a composition for prophylaxis or treatment of arthritis urica, or

for the manufacture of a composition for treatment of osteoarthrosis, or

for the manufacture of a composition for prophylaxis or treatment of a condition related to transplant rejection, such as for the manufacture of a composition for prophylaxis or treatment of a condition related to cornea transplantation, or

for the manufacture of a composition for prophylaxis or treatment of a condition related to a graft-versus-host reaction, or

for the manufacture of a composition for the treatment of Crohn's disease or ulcerative colitis, especially for the prevention of relapse or progression of Crohn's disease or ulcerative colitis

for the manufacture of a composition for the treatment of perniceous anemia or celiac disease, or

for the manufacture of a composition for prophylaxis or treatment of contact dermatitis, or

for the manufacture of a composition for prophylaxis or treatment of allergic/atopic dermatitis, or

for the manufacture of a composition for treatment of pemphigus vulgaris or pemphigoid, or

for the manufacture of a composition for treatment of a condition related to the function of the thyroid gland, in particular hyper- or hypofunctioning of said gland, e.g. thyroiditis (acute, subacute or chronic), including Hashimoto's disease (lymphocytic thyroiditis; lymphoid thyroiditis), Riedel's thyroiditis (chronic fibrous thyroiditis), de Quervain's thyroiditis (subacute granulomatous thyroiditis), subacute lymphocytic thyroiditis, Graves' disease, Graves'subacute thyroiditis or Graves' ophthalmopathy, or

for the manufacture of a composition for treatment of Simmonds' panhypopituitarism or

for the manufacture of a composition for treatment of a condition related to hypofunction of the adrenal glands, in particular Addison's disease, or

for the manufacture of a composition for treatment or prevention of replapse of a demyelinating disease, in particular multiple sclerosis, or

for the manufacture of a composition for treatment or prevention of relapse of sarcoidosis Boeck, Sjögren's syndrome, Reiter's syndrome, erythema nodosum, scleroderma or Bechet's disease, or

for the manufacture of a composition for treatment or prevention of relapse of polymyositis, polymyalgia rheumatica, myocarditis or systemic lupus erythematosis, or

for the manufacture of a composition for treatment or prevention of relapse of a condition related to vasculitis phenomena, e.g. polyarteritis nodosa, Wegener's granulomatosis, or giant-cell arteritis, or

for the manufacture of a composition for treatment or prevention of relapse of primary biliary cirrhosis or chronic active hepatitis, or

for the manufacture of a composition for treatment of aplastic anemia or idiopathic thrombocytopenic purpura, or

for the manufacture of a composition for treatment or prevention of relapse of a neoplastic disorder of the lymphoid tissue, e.g. B cell lymphoma or multiple myeloma, or

for the manufacture of a composition for treatment or prevention of relapse of a T lymphocyte proliferative disorder, e.g. mycosis fungoides or Sézary syndrome, or

for the manufacture of a composition for prophylaxis or treatment of septic shock caused by gram-negative bacteria, or

for the manufacture of a composition for prophylaxis or treatment of disseminated intravascular coagulation, or

for the manufacture of a composition for prophylaxis of arteriosclerosis, or

for the manufacture of a composition for prophylaxis or treatment of

a condition acute and chronic periodontal diseases, in particular periodontitis and periodontosis.

2. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of diabetes mellitus, in particular insulin-dependent diabetes mellitus (type 1), in particular for the prevention of progression of diabetes mellitus (type 1), especially substantially immediately after the first diagnostic establishment of diabetes mellitus, or for prophylaxis after establishment of being in a high risk group of developing diabetes mellitus (type 1).

3. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of progression of arthritis such as rheumatoid arthritis, psoriatic arthritis or Reiter arthritis substantially immediately after the first diagnostic establishment of arthritis.

4. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of septic shock caused by gram-negative bacteria.

5. The use of fusidic acid or a derivative thereof for the manufacture of a composition for treatment of Crohn's disease or ulcerative colitis, especially for the prevention of relapse or progression of Crohn's disease or ulcerative colitis.

6. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of a condition related to transplant rejection.

7. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of a condition related to a graft-versus-host reaction.

8. The use according to claim 6 of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of a condition related to cornea transplantation.

9. The use of fusidic acid or a derivative thereof for the manufacture of a composition for the treatment of endogenous uveitis.

10. The use of fusidic acid or a derivative thereof for the manufacture of a composition for the prevention of the inflammation after eye surgery such as cataract operation or laser surgery.

11. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of contact dermatitis.

12. The use of fusidic acid or a functional derivate thereof for the manufacture of a composition for treatment or prevention of relapse of systemic lupus erythematosis.

13. The use of fusidic acid or a functional derivate thereof for the manufacture of a composition for treatment or prevention of relapse of a neoplastic disorder of the lymphoid tissue such as B cell lymphoma or multiple myeloma.

14. The use of fusidic acid or a derivative thereof for use as an immunosuppressive drug.

15. The use according to any of claims 1-14 wherein the composition is a composition suitable for oral administration.

16. The use according to any of claims 1-14 wherein the composition is a composition suitable for rectal administration.

17. The use according to any of claims 1-14 wherein the composition is a composition suitable for parenteral administration.

18. The use according to any of claims 1-14 wherein the composition is a composition suitable for intraarticular administration.

19. The use according to any of claims 1-14 wherein the composition is a composition suitable for application to the skin.

20. The use according to any of claims 1-14 wherein the composition is a composition suitable for application to the eye.

21. The use according to any of claims 1-14 wherein the composition is a composition suitable for implantation administration.

22. The use of fusidic acid or a derivative thereof for the manufacture of a composition for the treatment of contact dermatitis, in particular for the manufacture of a composition for oral administration.

23. The use of fusidic acid or a derivative thereof for the manufacture of a composition for the topical treatment of contact dermatitis, in particular for the manufacture of a composition for topical administration to the skin.

24. The use of fusidic acid or a derivative thereof for the manufacture of a composition for treatment of Crohn's disease or colitis ulcerosa, in particular for the manufacture of an oral or rectal composition, or a composition for parenteral administration.

25. The use of fusidic acid or a derivative thereof for the manufacture of a composition for treatment of arthritis substantially immediately after the first diagnostic establishment of arthritis, in particular for the manufacture of a composition for oral use or for use as parenteral or intraarticular injections.

26. The use of fusidic acid or a derivative thereof for the manufacture of a composition for treatment of endogenous uveitis, in particular for the manufacture of a composition suitable for treatment of the eye, such as an eye drop composition, an eye ointment composition, an eye lotion composition or an injectable composition for intraocular or retroocular injection, or an oral composition.

27. The use of fusidic acid or a derivative thereof for the manufacture of a composition for the prevention of inflammation after eye surgery, in particular an oral composition, or a composition suitable

for treatment of the eye, such as an eye drop composition, an eye ointment composition, an eye lotion composition or an injectable composition for intraocular or retroocular injection.

28. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of a condition related to transplant rejection, in particular for the manufacture of a composition for oral or parenteral administration.

29. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of a condition related to a graft-versus-host reaction, in particular for the manufacture of a composition for oral or parenteral administration.

30. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of diabetes mellitus (type 1), in particular for the manufacture of a composition for oral or rectal use.

31. The use of fusidic acid or a derivative thereof for the manufacture of a composition for prophylaxis or treatment of septic shock caused by gram-negative bacteria, in particular for the manufacture of a composition for oral or parenteral administration.

32. The use of fusidic acid or a derivative thereof for the manufacture of a composition for treatment or prevention of relapse of systemic lupus erythematosis, in particular for the manufacture of a composition for oral or parenteral administration.

33. The use of fusidic acid or a derivative thereof for the manufacture of a composition for treatment or prevention of relapse of a neoplastic disorder of the lymphoid tissue such as B cell lymphoma or multiple myeloma, in particular for the manufacture of a composition for oral or parenteral administration.

34. The use according to any of the preceding claims wherein the fusidic acid or a derivative is used together with a ciclosporin (Cyclosporin A) or a derivative thereof or together with FK-506.

35. A method for substantially inhibiting a biological effect in a human related to a cytokine for prophylaxis or treatment of a condition related to disturbances of a cytokine system, such as

for prophylaxis or treatment of diabetes mellitus, in particular insulin-dependent diabetes mellitus (type 1), in particular for the prevention of progression of diabetes mellitus (type 1), especially substantially immediately after the first diagnostic establishment of diabetes mellitus, or for prophylaxis after establishment of being in a high risk group of developing diabetes mellitus (type 1), or

for the treatment of endogenous uveitis, or

for the prevention of the inflammation after eye surgery such as cataract operation or laser surgery, or

for treatment of progression of arthritis such as rheumatoid arthritis, psoriatic arthritis or Reiter arthritis substantially immediately after the first diagnostic establishment of arthritis, or

for prophylaxis or treatment of arthritis urica, or

for treatment of osteoarthrosis, or

for prophylaxis or treatment of a condition related to transplant rejection, such as for prophylaxis or treatment of a condition related to cornea transplantation, or

for prophylaxis or treatment of a condition related to a graft-versus-host reaction, or

for the treatment of Crohn's disease or ulcerative colitis, especially for the prevention of relapse or progression of Crohn's disease or ulcerative colitis

for the treatment of perniceous anemia or celiac disease, or

for prophylaxis or treatment of contact dermatitis, or

for prophylaxis or treatment of allergic/atopic dermatitis, or

for treatment of pemphigus vulgaris or pemphigoid, or

for treatment of a condition related to the function of the thyroid gland, in particular hyper- or hypofunctioning of said gland, e.g. thyroiditis (acute, subacute or chronic), including Hashimoto's disease (lymphocytic thyroiditis; lymphoid thyroiditis), Riedel's thyroiditis (chronic fibrous thyroiditis), de Quervain's thyroiditis (subacute granulomatous thyroiditis), subacute lymphocytic thyroiditis, Graves' disease, Graves'subacute thyroiditis or Graves' ophthalmopathy, or

for treatment of Simmonds' panhypopituitarism or

for treatment of a condition related to hypofunction of the adrenal glands, in particular Addison's disease, or

for treatment or prevention of replapse of a demyelinating disease, in particular multiple sclerosis, or

for treatment or prevention of relapse of sarcoidosis Boeck, Sjögren's syndrome, Reiter's syndrome, erythema nodosum, scleroderma or Bechet's disease, or

for treatment or prevention of relapse of polymyositis, polymyalgia rheumatica, myocarditis or systemic lupus erythematosis, or

for treatment or prevention of relapse of a condition related to vasculitis phenomena, e.g. polyarteritis nodosa, Wegener's granulomatosis, or giant-cell arteritis, or

for treatment or prevention of relapse of primary biliary cirrhosis or chronic active hepatitis, or

for treatment of aplastic anemia or idiopathic thrombocytopenic purpura, or

for treatment or prevention of relapse of a neoplastic disorder of the lymphoid tissue, e.g. B cell lymphoma or multiple myeloma, or

for treatment or prevention of relapse of a T lymphocyte proliferative disorder, e.g. mycosis fungoides or Sézary syndrome, or

for prophylaxis or treatment of septic shock caused by gram-negative bacteria, or

for prophylaxis or treatment of disseminated intravascular coagulation, or

for prophylaxis of arteriosclerosis, or

for prophylaxis or treatment of a condition acute and chronic periodontal diseases, in particular periodontitis and periodontosis,

the method comprising administering to a human in need thereof an effective amount of fusidic acid or a derivative thereof.

36. A method of treating diabetes mellitus, in particular insulin-dependent diabetes mellitus (type 1) which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

37. A method of preventing a progression of diabetes mellitus, in particular insulin-dependent diabetes mellitus (type 1) which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

38. A method according to claim 37 wherein the effective amount of fusidic acid or a derivative thereof is administered substantially immediately after the first diagnostic establishment of diabetes mellitus.

39. A method of prophylactic treatment of diabetes mellitus (type 1) after establishment of being in a high risk group of developing diabetes mellitus (type 1).

40. A method of treatment or prevention of the inflammation after eye surgery, which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

41. A method of treating endogeneous uveitis, which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

42. A method of treating arthritis substantially immediately after the first diagnostic establishment of arthritis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

43. A method of treating arthritis urica which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

44. A method of treating osteoarthrosis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

45. A method of treating a condition related to transplant rejection which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

46. A method of treating a condition related to a graft-versus-host reaction which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

47. A method of treating a condition cornea transplantation, which

method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

48. A method of treating or preventing a relapse of Crohn's disease or ulcerative colitis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

49. A method of treating pernicious anemia or celiac disease which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

50. A method of treating contact dermatitis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

51. A method of treating allergic/atopic dermatitis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

52. A method of treating pemphigus vulgaris or pemphigoid which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

53. A method of treating a condition which is related to the function of the thyroid gland, in particular hyper- or hypofunctioning of said gland, e.g. thyroiditis (acute, subacute or chronic), including Hashimoto's disease (lymphocytic thyroiditis; lymphoid thyroiditis), Riedel's thyroiditis (chronic fibrous thyroiditis), de Quervain's thyroiditis (subacute granulomatous thyroiditis), subacute lymphocytic thyroiditis, Graves' disease, Graves'subacute thyroiditis, Graves' ophthalmopathy or Simmond's panhypopituitarism which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

54. A method of treating a condition which is related to the hypofunction of the adrenal glands, in particular Addison's disease, which method comprises administering to a human in need of such

treatment an effective amount of fusidic acid or a derivative thereof.

55. A method of treating or preventing relapse of a demyelinating disease, in particular multiple sclerosis, which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

56. A method of treating or preventing relapse of sarcoidosis Boeck, Sjögren's syndrome, Reiter's syndrome, erythema nodosum, scleroderma or Bechet's disease which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

57. A method of treating or preventing relapse of polymyositis, polymyalgia rheumatica, myocarditis or systemic lupus erythematosis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

58. A method of treating or preventing a relapse of a condition related to vasculitis phenomena, e.g. polyarteritis nodosa, Wegener's granulomatosis, or giant-cell arteritis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

59. A method of treating or preventing a relapse of primary biliary cirrhosis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

60. A method of treating aplastic anemia or idiopathic thrombocytopenic purpura which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

61. A method of treating or preventing a relapse of a neoplastic disorder of the lymphoid tissue, e.g. B-cell lymphoma or multiple

myeloma, which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

62. A method according to claim 62 wherein disorder is multiple myeloma.

63. A method of treating or preventing a relapse of a T-lymphocyte proliferative disorder, e.g. mycosis fungoides or Sézary syndrome which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

64. A method of treating or preventing a condition of septic shock caused by gram-negative bacteria, which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

65. A method of treating disseminated intravascular coagulation which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

66. A method of prophylactic treatment of arteriosclerosis which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

67. A method of treating a condition related to acute and chronic periodontal diseases, in particular periodontitis and periodontosis, which method comprises administering to a human in need of such treatment an effective amount of fusidic acid or a derivative thereof.

68. A method according to any of claims 36-68 wherein the fusidic acid is administered orally, preferably in an amount from about 1 mg to about 75 mg per kg body weight per day.

69. A method according to any of claims 36-68 wherein the fusidic

acid is administered rectally, preferably in an amount from about 1 mg to about 100 mg per kg body weight per day.

70. A method according to any of claims 36-68 wherein the fusidic acid is administered topically to the skin in an effective amount.

71. A method according to any of claims 36-68 wherein the fusidic acid is administered to the eye, preferably in an amount from about 0.1 mg to about 50 mg per kg body weight per day.

72. A method according to any of claims 36-68 wherein the fusidic acid is administered parenterally, preferably in an amount from about 0.1 mg to about 50 mg per kg body weight per day, most preferably in an amount from about 1 mg to about 20 mg per kg body weight per day.

73. A method according to any of claims 36-68 wherein the fusidic acid is administered intraarticularly, preferably in an amount from about 0.1 mg to 20 mg per kg body weight per day.

74. The use according to any of claims 1-35 wherein the derivative of fusidic acid is selected from the group consisting of dehydrofusidic acid; 3,11-didehydrofusidic acid; 24,25-dihydrofusidic acid; 17,20-24,25-tetrahydrofusidic acid; 17,20-24,25-tetrahydrofusidic acid and their corresponding 3-acetates (especially conjugated with glycine or taurine); and 3-0-acetyl-16-epideacetylfusidic acid.

75. A method according to any of claims 36-68 wherein the derivative of fusidic acid is selected from the group consisting of dehydrofusidic acid; 3,11-didehydrofusidic acid; 24,25-dihydrofusidic acid; 17,20-24,25-tetrahydrofusidic acid; 17,20-24,25-tetrahydrofusidic acid and their corresponding 3-acetates (especially conjugated with glycine or taurine); and 3-0-acetyl-16-epideacetylfusidic acid.

Fig. 1

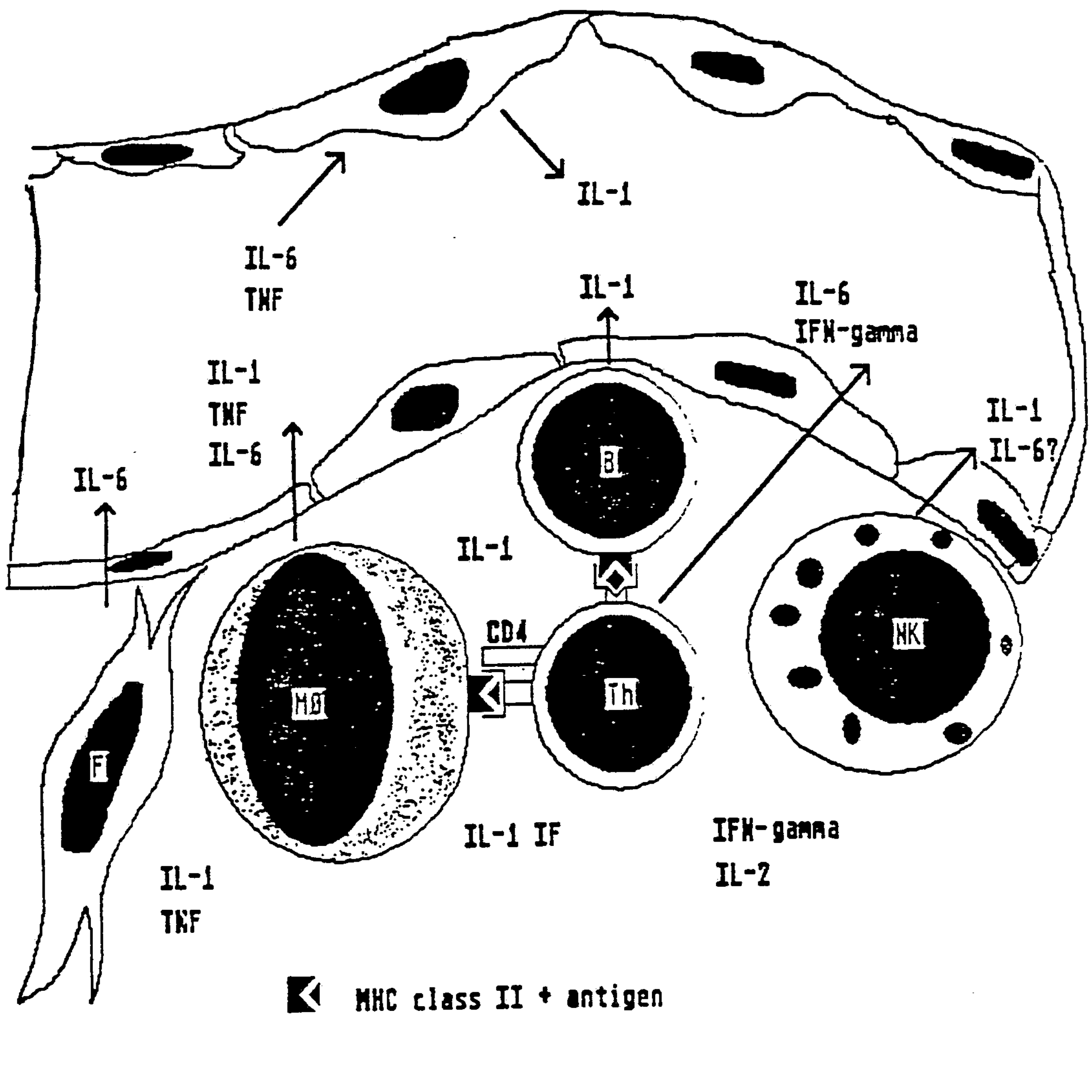
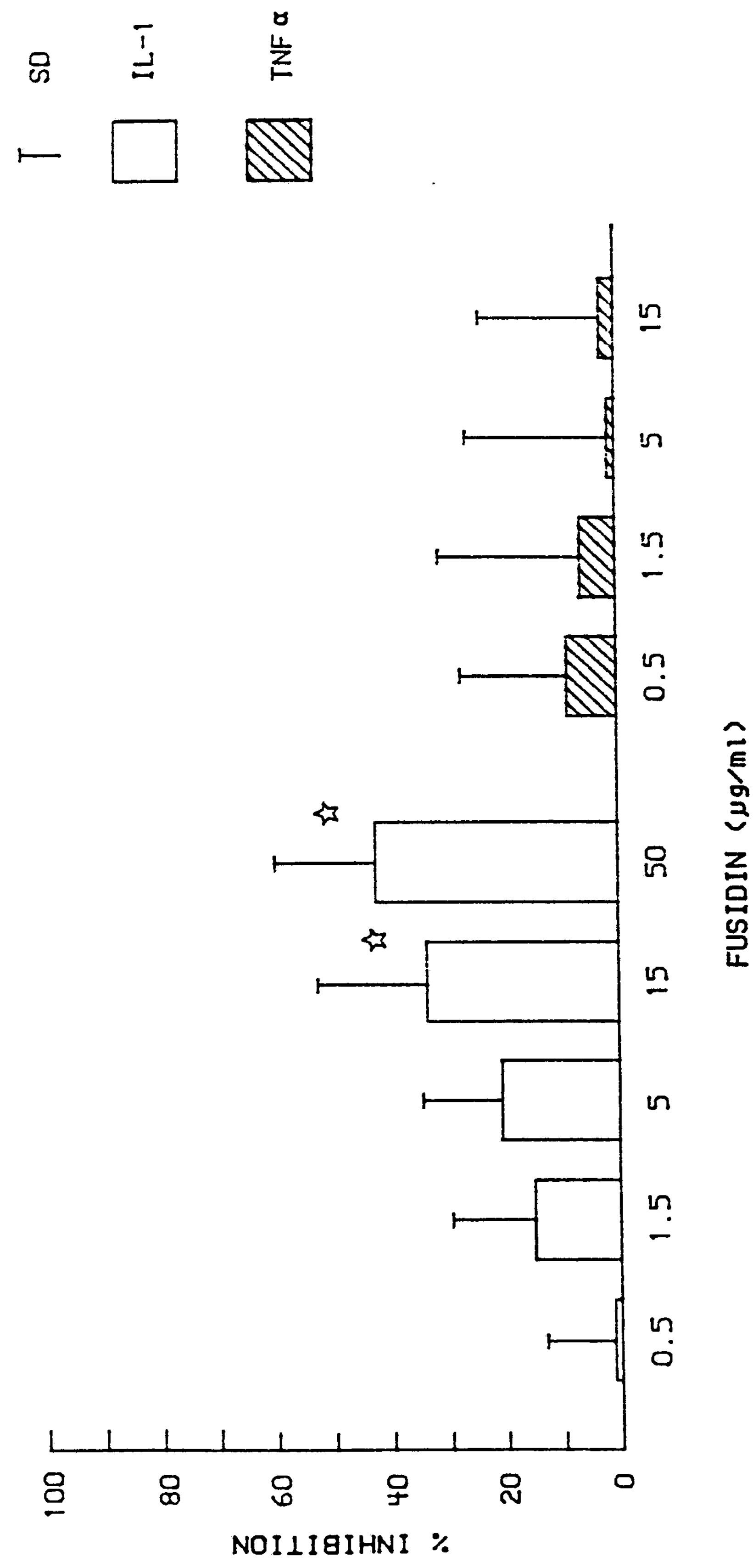

IL-1
IL-6
TNF
IL-1
IL-6
IFN-gamma
IL-1
TNF
IL-6
IL-1
IL-6?
IL-6
B
IL-1
CD4
MØ
Th
NK
F
IL-1 IF
IFN-gamma
IL-2
IL-1
TNF
MHC class II + antigen

Fig. 2


SD
IL-1
TNF α
% INHIBITION
100
80
60
40
20
0
0.5
1.5
5
15
50
0.5
1.5
5
15
FUSIDIN (μg/ml)

3/13

Fig. 3

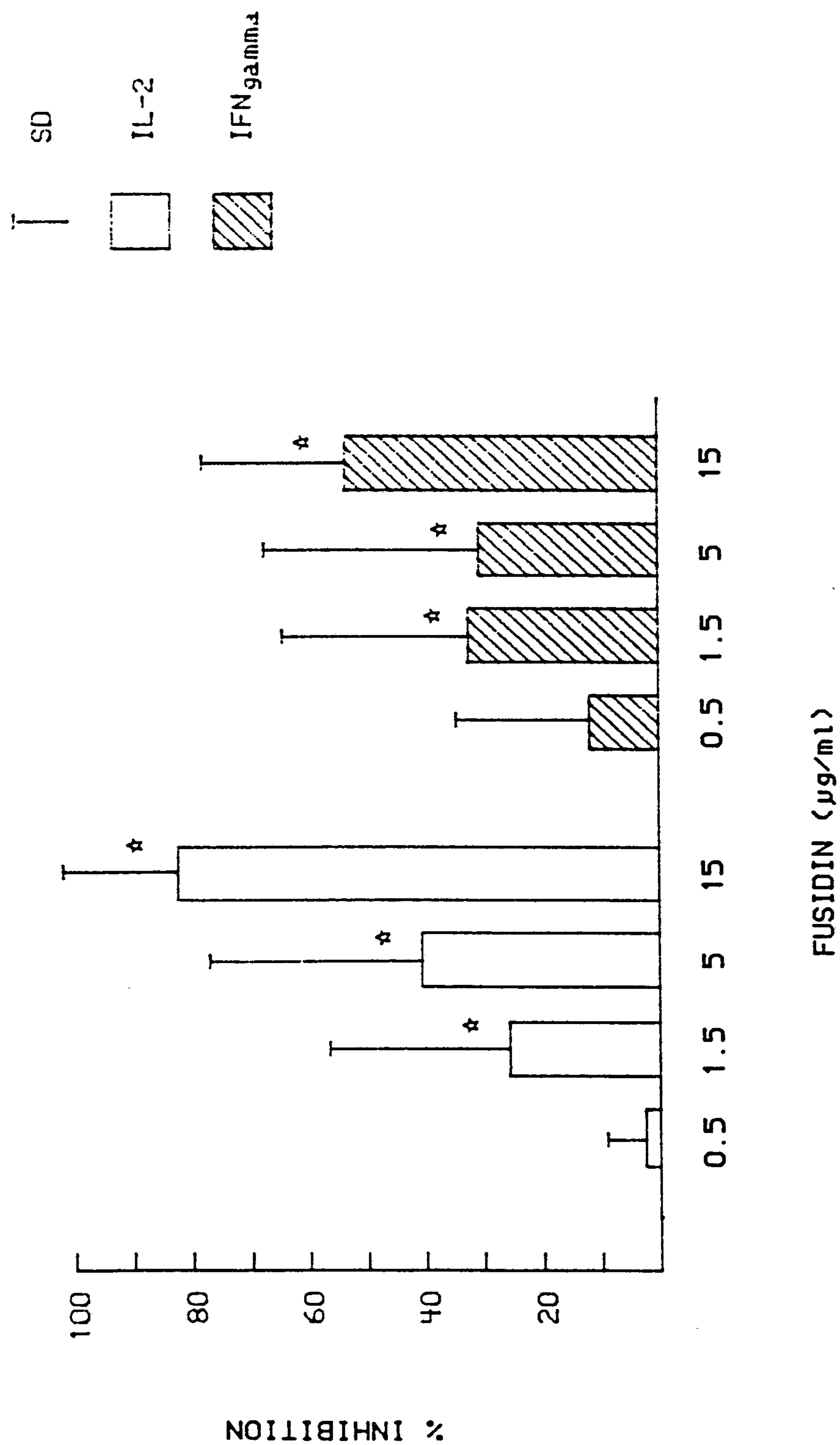
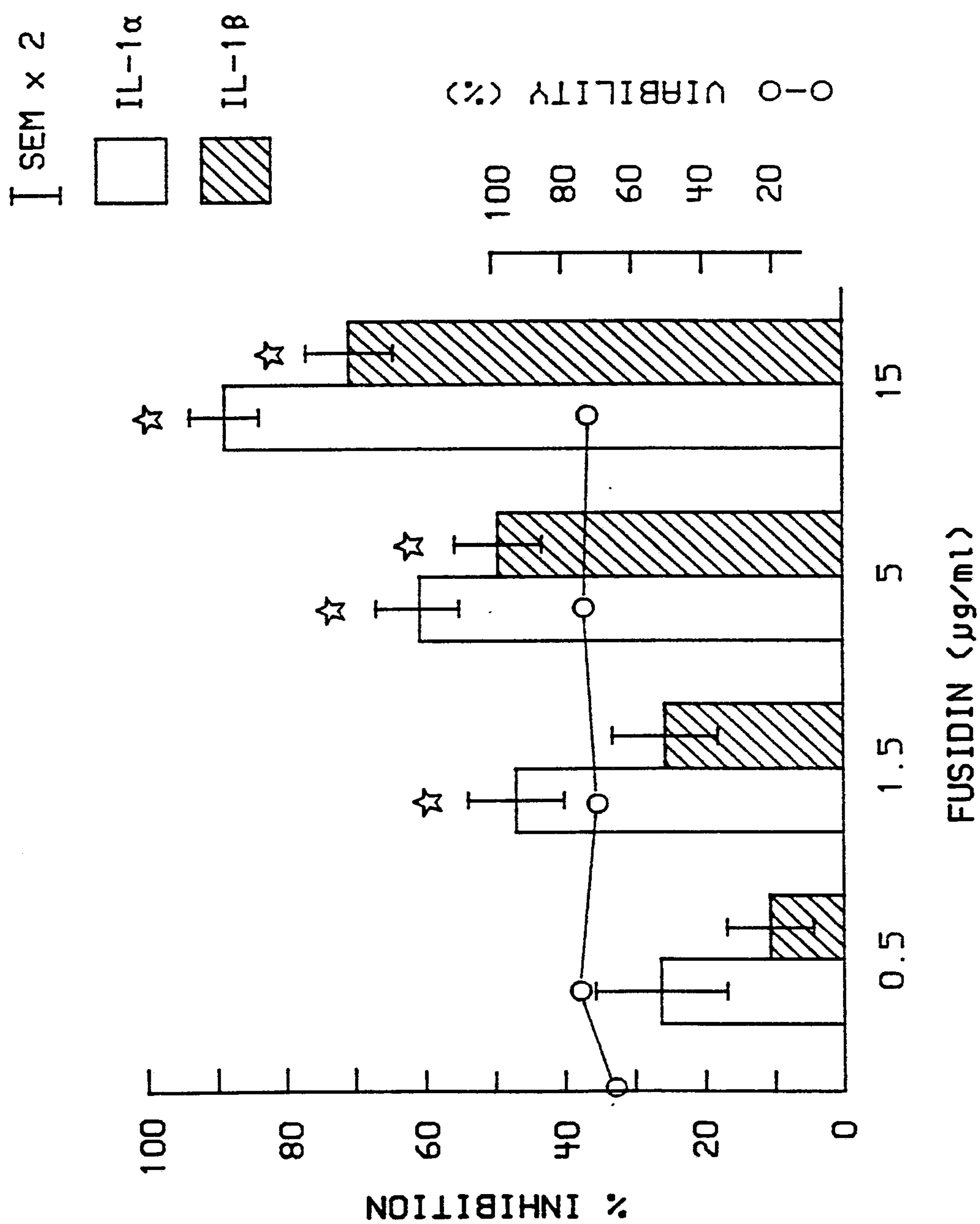

SD
IL-2
IFNgamma
% INHIBITION
100
80
60
40
20
0.5
1.5
5
15
0.5
1.5
5
15
FUSIDIN (μg/ml)

Fig. 4


SEM x 2
IL-1α
IL-1β
O–O VIABILITY (%)
100
80
60
40
20
% INHIBITION
100
80
60
40
20
0
0.5
1.5
5
15
FUSIDIN (µg/ml)

5/13

Fig. 5

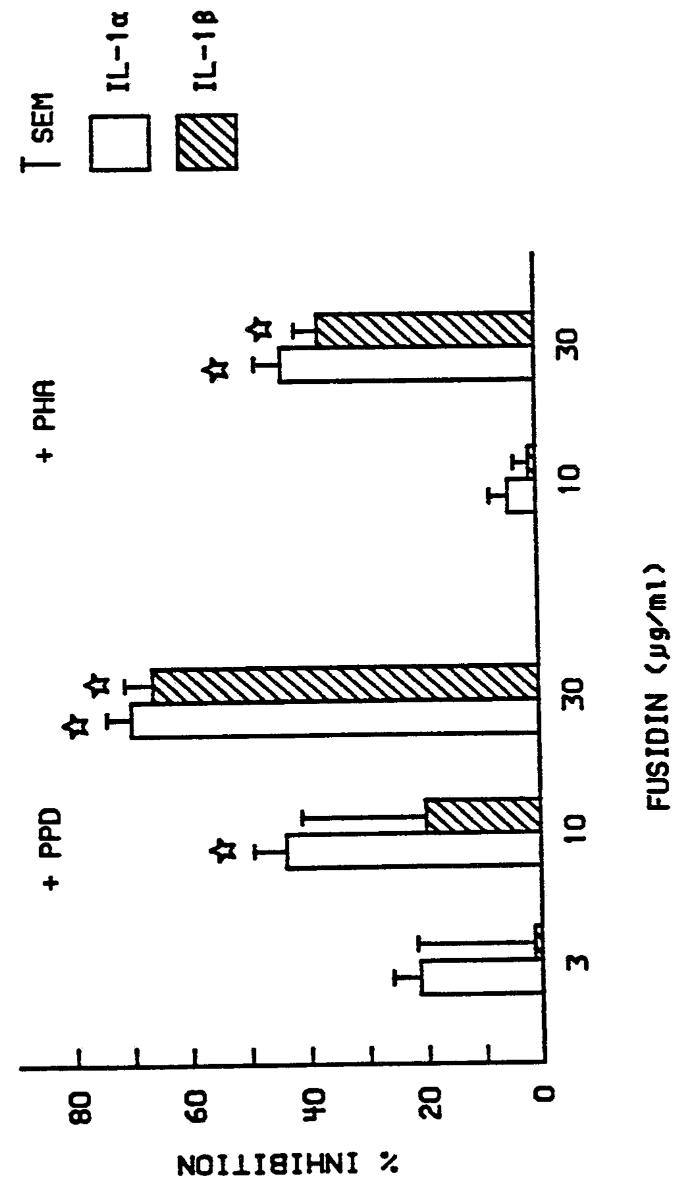
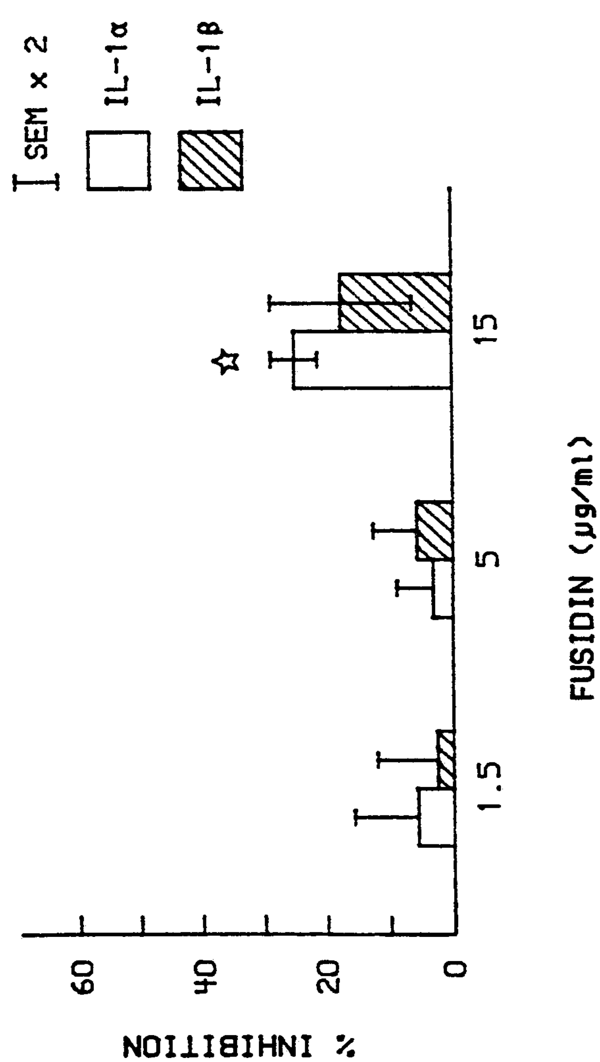

SEM
IL-1α
IL-1β
+ PPD
+ PHA
% INHIBITION
0
20
40
60
80
3
10
30
10
30
FUSIDIN (μg/ml)

Fig. 6



7/13

Fig. 7

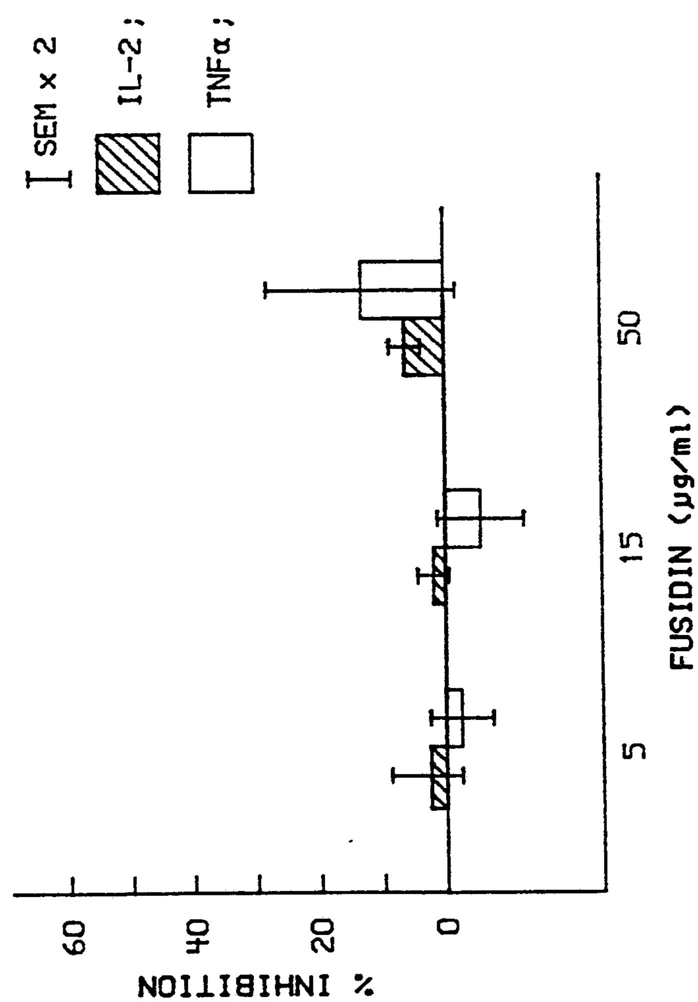


Fig. 8

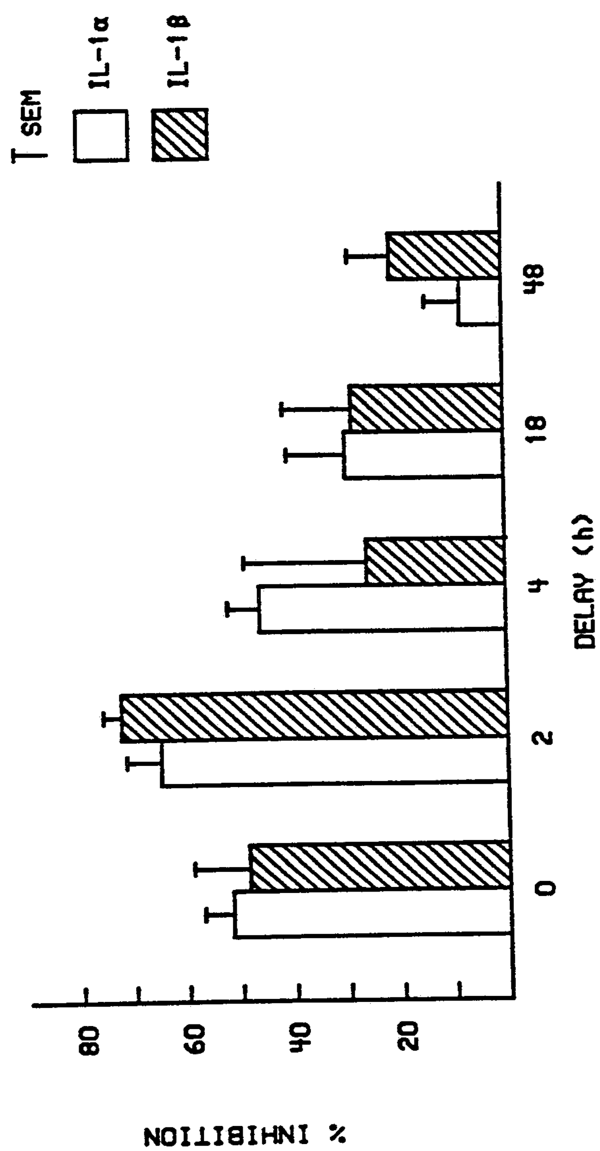


Fig. 9

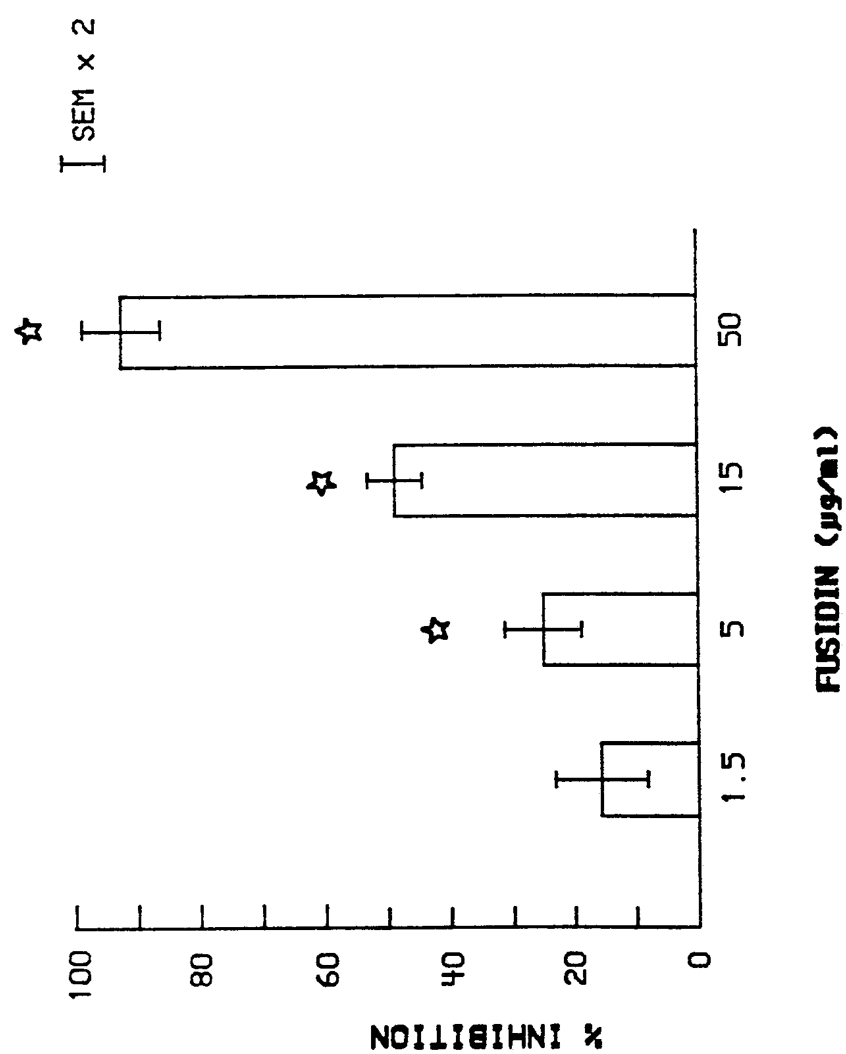


Fig. 10

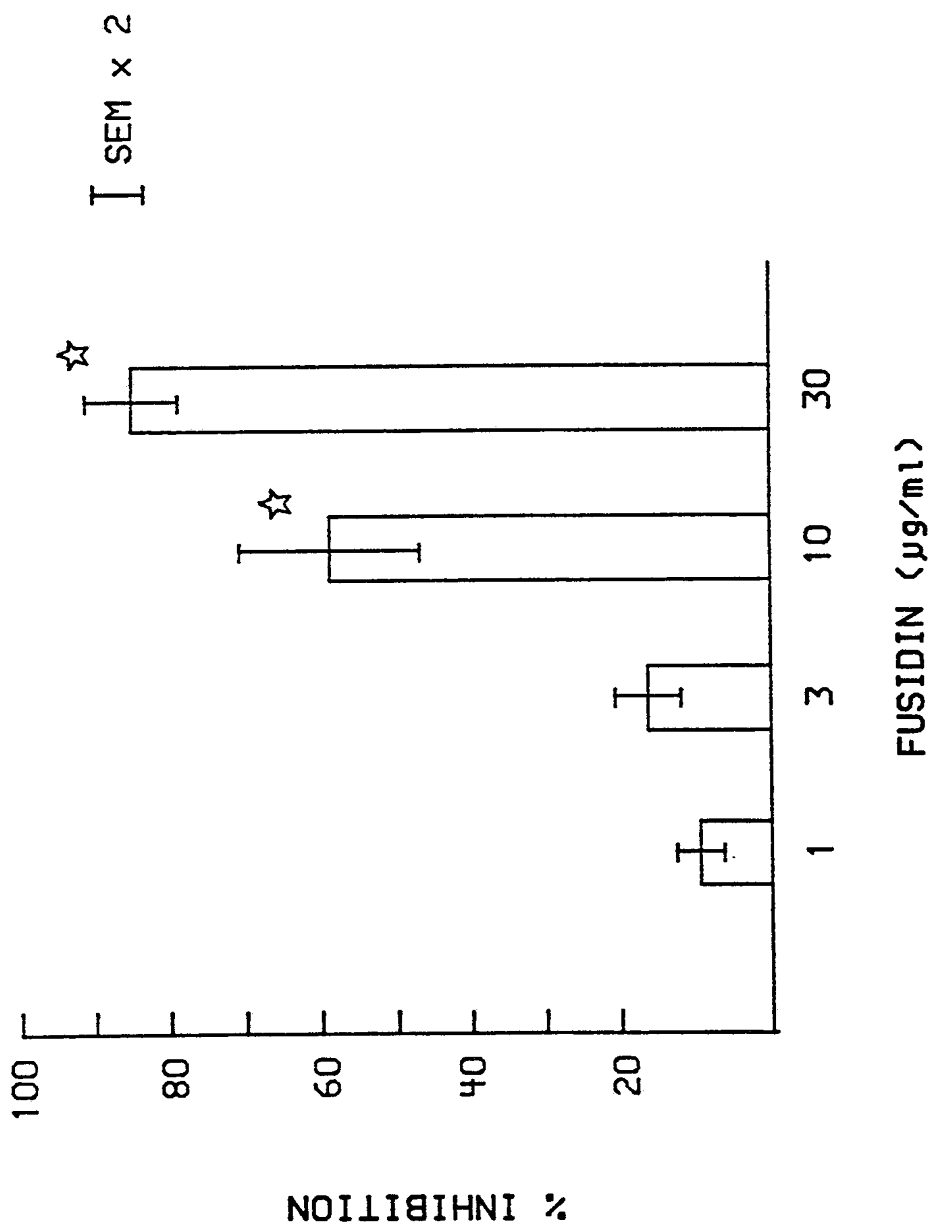
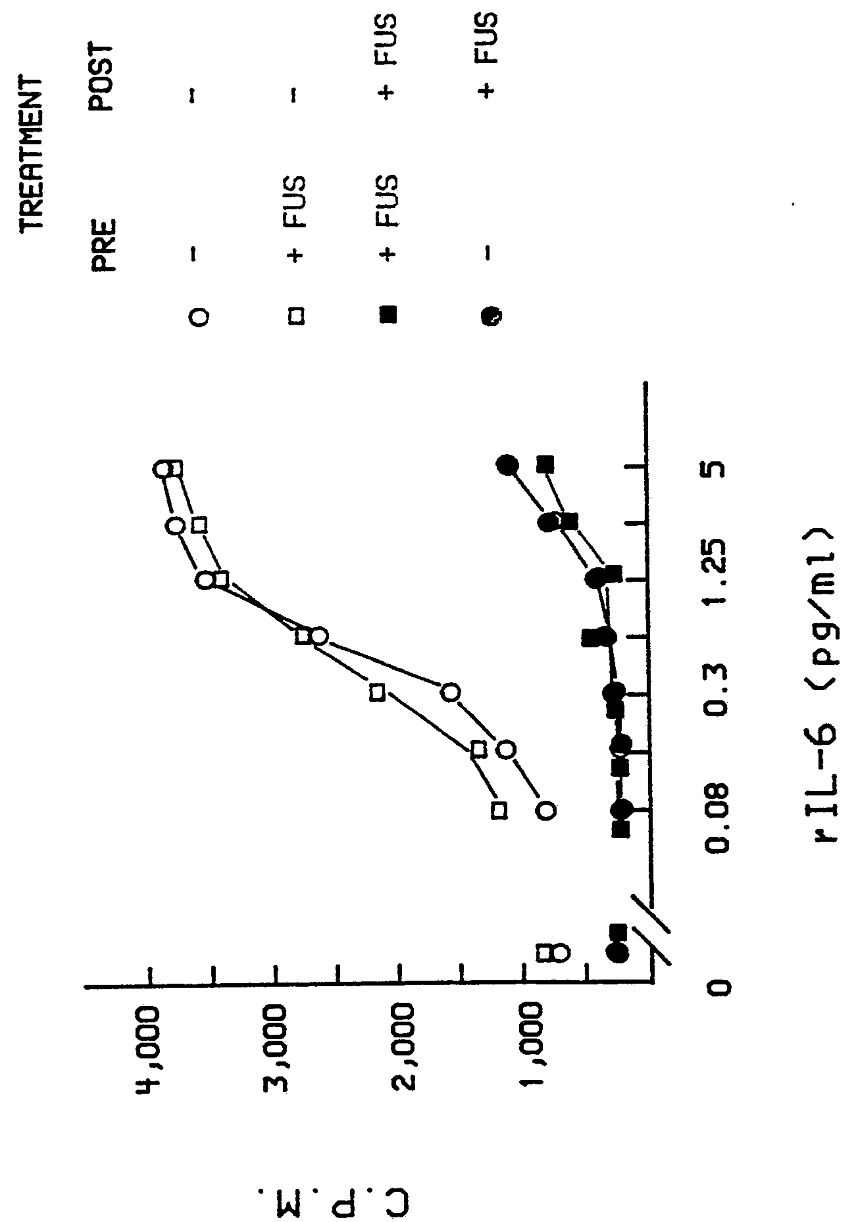


Fig. 11



TREATMENT
PRE POST
○ – –
□ + FUS –
■ + FUS + FUS
● – + FUS
C.P.M.
4,000
3,000
2,000
1,000
0
0.08
0.3
1.25
5
rIL-6 (pg/ml)

12/13

Fig. 12

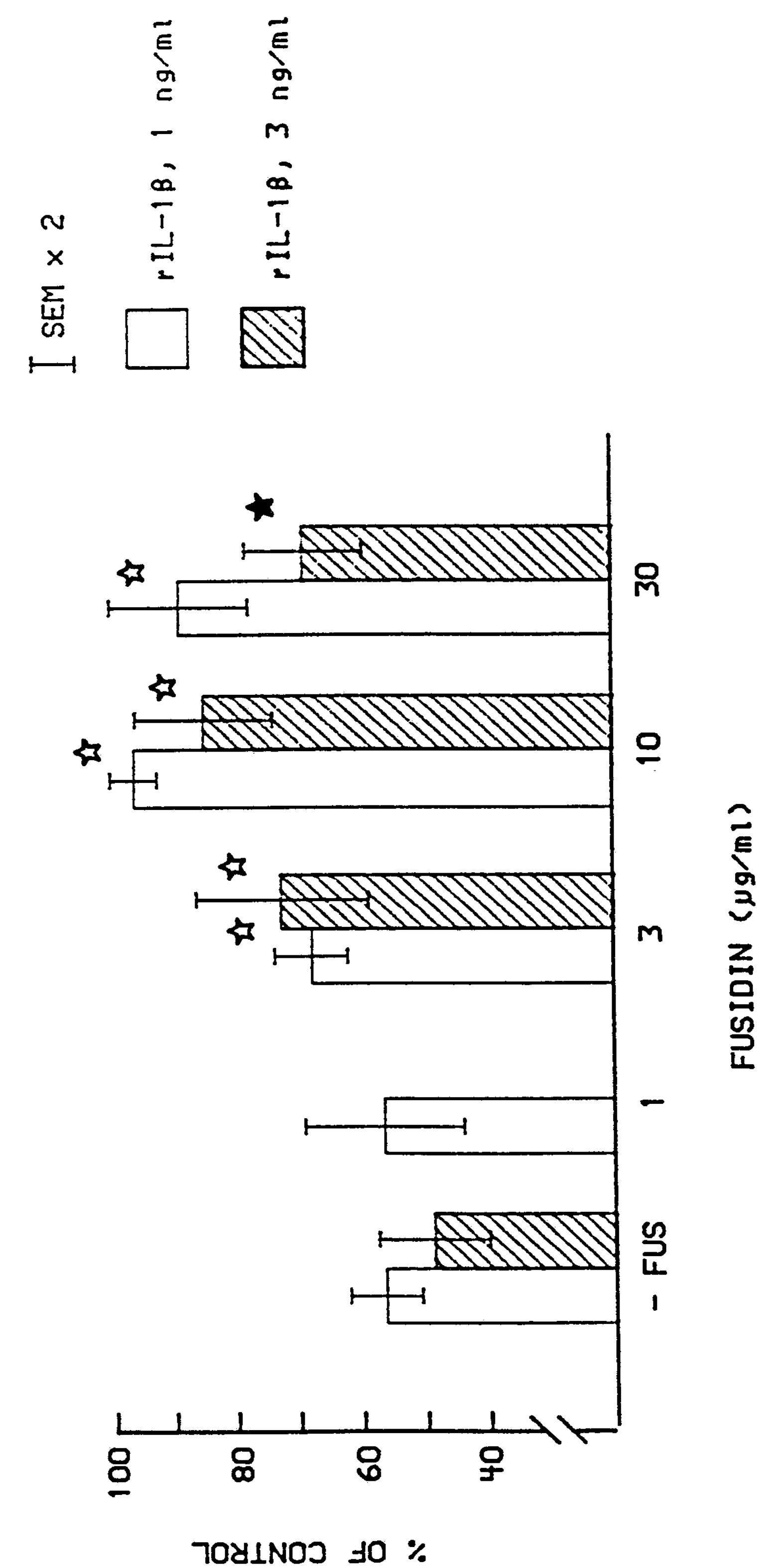
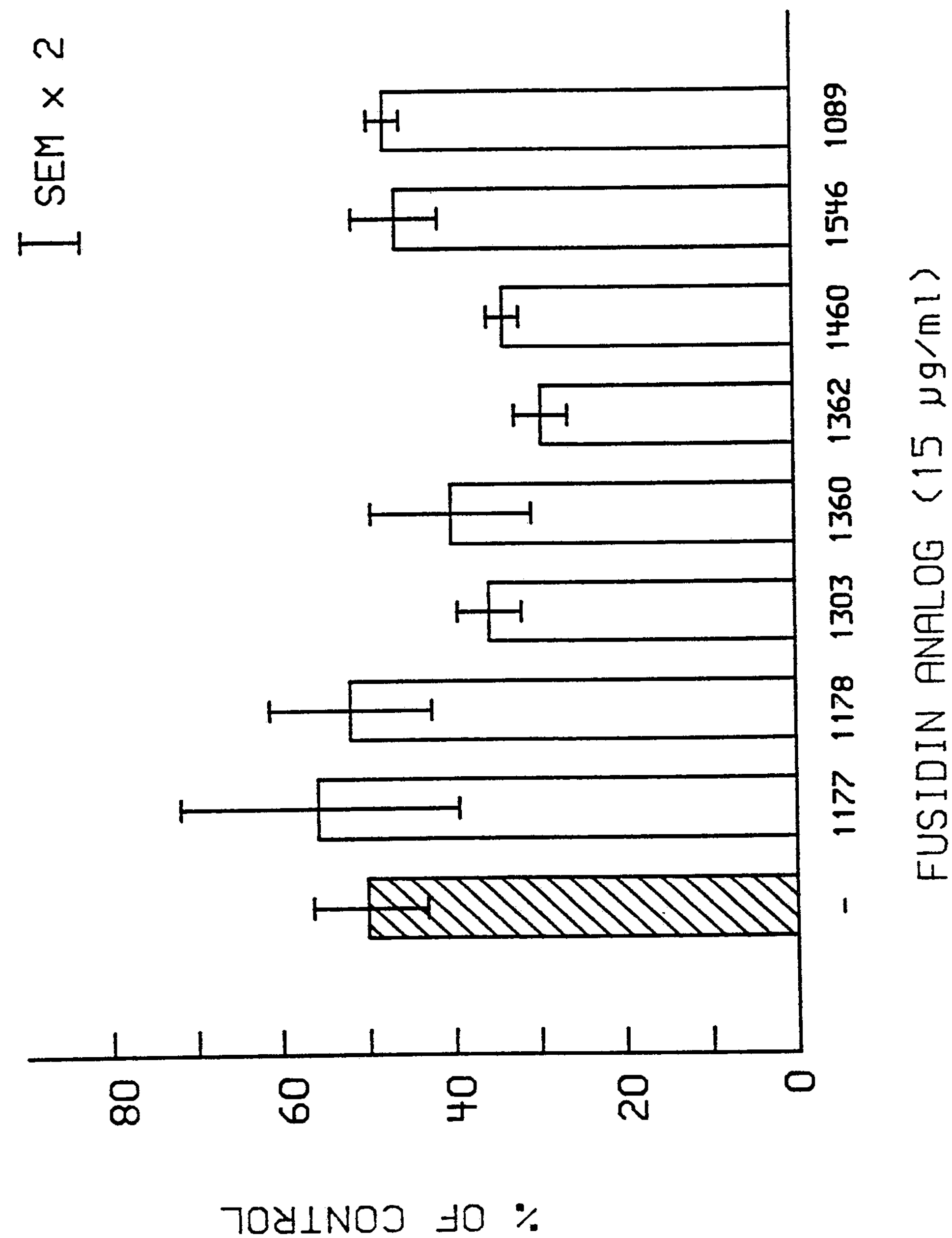

SEM × 2
rIL-1β, 1 ng/ml
rIL-1β, 3 ng/ml
% OF CONTROL
100
80
60
40
– FUS
1
3
10
30
FUSIDIN (μg/ml)

Fig. 13


SEM × 2
% OF CONTROL
0
20
40
60
80
-
1177
1178
1303
1360
1362
1460
1546
1089
FUSIDIN ANALOG (15 µg/ml)

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 89/00254

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 31/575, C 07 J 13/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 07 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

Database search: CA, Medline
SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	US, A, 4004004 (WELF VON DAEHNE) 18 January 1977, see the whole document --	1-34, 76
X	US, A, 3920817 (WAGN OLE GODTFREDSEN) 18 November 1975, see the whole document --	1-34, 76
X	EP, A2, 0285285 (PUBLIC HEALTH LABORATORY SERVICE BOARD) 5 October 1988, see esp. page 2 lines 48-53 --	1-34, 76
X	The Lancet, 25 September 1971, Norman Jackson: "Fusidic acid treatment in psoriasis ", see page 712 --	1-34, 76

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25th January 1990	1990 -02- 01
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Eva Johansson Eva Johansson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Contact dermatitis., Vol. 8, No. 6, November 1982 Anton C. de Groot: "Contact allergy to sodium fusidate ", see page 429 --	1-34, 76
X	The British Journal of Clinical Practice, Vol. 27, No. 3, March 1973 Aziz Kurwa et al: "Preliminary Clinical Trial of Fusidic Acid in Psoriasis ", see page 92 - page 94 --	1-34, 76
X	British Journal of Ophthalmology, Vol. 72, 1988 Peter B. Taylor et al: "Effect of preoperative fusidic acid on the normal eyelid and conjunctival bacterial flora ", see page 206 - page 209 see esp. page 207 --	1-34, 76
X	Scand. J. Plast Reconstr. Surg., Vol. 6, 1972 Anne Marie Johansen and Bent Sørensen: "Treatment of Donor Sites: A Controlled Trial with Fucidin Gauze ", see page 47 - page 50 --	1-34, 76
X	Scand. J. Rheumatology, Vol. 1, 1972 Shridhar D. Deodhar et al: "Penetration of Sodium Fusidatel (Fucidin) in the Synovial Cavity ", see page 33 - page 39 --	1-34, 76

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 35-75, because they relate to subject matter not required to be searched by this Authority, namely:

   Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods (see article 17(2) and Rule 39(iv)).

2. [X] Claim numbers 1-34, 76, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   The wording of the claims with different biological activities do not clearly define one solution of one technical problem. If there are more than one technical problem the search has not been complete.

   The search report has been based on the assumption that there is only one technical problem and one solution. As the problem is solved .../...

3. [ ] Claim numbers.........., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED

through the cited references they are relevant to all the claims.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 89/00254

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
08/11/89

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4004004	18/01/77	BE-A- 833313	11/03/76
		NL-A- 7510772	16/03/76
		FR-A- 2284330	09/04/76
		DE-A- 2540591	01/04/76
		LU-A- 73372	13/08/76
		AU-D- 84649/75	17/03/77
		GB-A- 1490852	02/11/77
		JP-A- 51054543	13/05/76
		SE-A- 7510147	15/03/76
US-A- 3920817	18/11/75	NL-A- 7411402	17/03/75
		BE-A- 819891	13/03/75
		DE-A- 2443431	27/03/75
		AU-D- 73255/74	18/03/76
EP-A2- 0285285	05/10/88	AU-D- 13216/88	15/09/88
		JP-A- 63301823	08/12/88